MORPHOMETRICS, CHARACTERIZATION AND SEEDLING EMERGENCE STUDIES OF A COLLECTION OF RUSSELL LUPIN (*Lupinus polyphyllus x Lupinus arboreus*) GENOTYPES IN CANTERBURY

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In a field experiment, a germplasm collection of 18 Russell lupin accessions, obtained from the USSR (5), New Zealand (5), Poland (3), Germany (3), Portugal (1), and the United Kingdom (1), was grown on a Wakanui silt loam soil at Lincoln (43° 38'S.) in Canterbury. The morphology, growth, performance, and flowering characteristics of the accessions were studied. Canonical variate analysis was used to characterize the germplasm collection on morphological and agronomic attributes.

Seedling emergence of the Russell lupin accession (Connie), which has considerable agronomic potential for sheep grazing in the hill and high country of New Zealand, was evaluated for response to scarification method, depth of sowing, and temperature in a series of laboratory and glasshouse experiments.

The scatterplot of the canonical variate means of accessions facilitated the identification of four distinct groups of accessions in the germplasm collection. Characterization of the genotypes using univariate statistics, or by simple visual observation was extremely difficult. The groups and their main characteristics are described and discussed. In terms of overall relationships, the Russell lupin should be regarded as a plant which is highly variable in its morphology, growth, performance, and flowering characteristics.

A latitude of origin pattern of variation was represented along the first canonical axis, which accounted for 35.2% of the between-accession variation. Along this axis, all, but one, accessions from New Zealand and the accession from Portugal were grouped together as opposed to the group of accessions from northern Europe. Flowering before exposure to cold and/or short days, days to first flower, dry matter yield in autumn and leaflet dimension appear to be the most discriminatory attributes in separating accessions along the first canonical axis.

Along the second canonical axis, which accounted for a further 16.4% of the between-accession variation, there was wide variation in plant size and spring dry matter yield. Two New Zealand accessions (HN & ON), with a higher dry matter yield than the mean of the 18 accessions were identified, although these accessions show different flowering responses, perhaps, to cool conditions along the first canonical axis. Conversely, all accessions from the USSR were

vernalization requiring, high latitude types, and had a low dry matter yield in both autumn and spring.

Connie lupin (CN), used as the control, was an early flowering, non-vernalization requiring, low latitude type, and was of medium plant height and had an intermediate dry matter yield.

The wide diversity within and among the groups should provide good opportunity for future breeding and agronomic work on Russell lupin in New Zealand.

A high proportion of freshly harvested Russell lupin seed is hard. Laboratory and glasshouse studies showed that the germination and emergence of freshly harvested Russell lupin seed can be improved by scarification using chipping or concentrated sulphuric acid (36N). However, a large proportion of abnormal seedlings (36% of the seeds sown) was produced by chipping. Acid scarification for 30 or 45 minutes produced more than 75% normal seedlings.

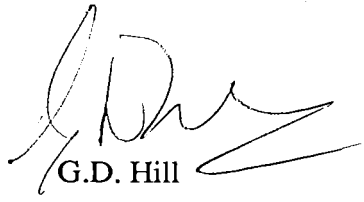
In a glasshouse study, depth of sowing affected both emergence and seedling vigour of Russell lupin. Emergence was best (92%) when the seed was sown at 1 cm below the soil surface. The best seedling growth (seedling height, root length, oven dry weight of tops and roots) was also obtained by sowing at 1 cm. However, sowing at 2 cm gave results which were not statistically different ($p<0.05$) from the 1 cm sowing.

In a controlled temperature study where depth of sowing was constant, final emergence of Russell lupin was reduced considerably at 25 ^{o}C. Emergence was best (92%) at 20 ^{o}C, however, temperature between 10 - 20 ^{o}C did not limit the final emergence of Russell lupin. The lowest critical temperature for seed germination and seedling emergence is less than 10 ^{o}C.

KEY WORDS Russell lupin, perennial lupin, legume, morphology, germplasm characterization, canonical variate analysis, emergence, scarification, sowing depth, temperature effects

CERTIFICATE

This is to certify that the experimental work reported in this thesis was planned, executed and described by Mesfin Tesfaye, under my direct supervision.



G.D. Hill

Supervisor

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CHAPTER ONE

GENERAL INTRODUCTION

Factors limiting production in the extensive grasslands of New Zealand's hill and high country include low soil fertility (N, P, S, Mo), soil acidity, low temperatures, seasonal soil moisture deficits and low dry matter production by resident species (Sinclair and McIntosh, 1983; Chapman and MacFarlane, 1985; Scott *et al.*, 1985). Until recently, the nutritional quality, dry matter production and stock carrying capacity of hill country pastures had been improved by the introduction of legumes following the correction of soil nutrient deficiencies (White, 1984; 1989; Chapman and MacFarlane, 1985; Scott *et al.*, 1985). Generally, introduced pasture species have a higher nutrient demand than resident plant species (Chapin, 1980), and their greater potential yield is only realised when nutrients are applied. However, recent escalation of development costs, together with a decline in returns from meat production, has led hill country farmers and scientists to consider alternative methods of increasing animal production.

One alternative approach may be to introduce pasture species that grow at low temperatures and at low soil pH, which require minimum amounts of initial and maintenance fertiliser, nodulate readily and thus improve soil nitrogen status and finally produce a high yield of quality forage which is acceptable to livestock. One such alternative plant may be the Russell lupin (*Lupinus polyphyllus X Lupinus arboreus*).

The Russell lupin, a common garden ornamental, was selected and bred in the late 1920s by, George Russell (1857 -1951), a Yorkshire gardener (Anderson, 1959, Hadfield, 1960, Gorer, 1970). The plant is highly attractive from an aesthetic point of view, especially when flowering, and has gained widespread horticultural recognition. In New Zealand, the Russell lupin was deliberately introduced as a garden plant and has escaped to invade river beds and road verges in many areas of the South Island. Recent studies in New Zealand have shown that Russell lupin grows better where plant nutrients, especially P, are scarce than the extensively utilized *Trifolium* spp. (Fitzgerald, 1980; Davis, 1981a; 1981b; Scott and Covacevich, 1987), and has considerable agronomic potential as a grazing plant for sheep on high country soils (Scott and Covacevich, 1987).

Although the Russell lupin was the highest yielding species at zero or low levels of applied phosphorus fertiliser (Davis, 1981b; Scott and Covacevich, 1987), and produced a large amount of leaf and fine stem biomatter (453 g DM m^{-2} after four months) (McKendry, 1987), this forage was not always acceptable to grazing sheep (Scott and Covacevich, 1987). It is possible that there may be other lines of Russell lupin that have greater animal acceptability.

There is virtually no published information on the establishment, growth and genetic diversity of Russell lupins. A series of laboratory and glasshouse studies and a field experiment were initiated to improve the information base on this plant. The aim of this research project was to obtain data on Russell lupin for use in future agronomic and breeding studies on this plant. The general objectives of this research project were:-

1. to record and describe the morphology, growth, and performance of Russell lupin accessions;
2. to characterize the genetic resource, represented by the accessions of Russell lupins, collected from different countries;
3. to identify and select Russell lupin accessions with increased dry matter production so that these can be examined in more detail;
4. to investigate different scarification methods to obtain optimum seed germination and emergence of normal Russell lupin seedlings;
5. to examine the effect of sowing depth on seedling emergence and seedling vigour of Russell lupin;
6. to investigate the effect of temperature on the rate and total emergence of Russell lupin.

Objectives 1, 2 and 3 were studied in a field experiment. Scarification effects and the effect of sowing depth were studied in a glasshouse, and the effect of temperature on seedling emergence was investigated in temperature controlled cabinets.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 TAXONOMY AND GENETICS OF *LUPINUS*

2.1.1 Taxonomy

The genus *Lupinus* L. belongs to the sub-family Papilionoideae of the family Leguminosae (Everett, 1981; Beltekey and Kovacs, 1984). Because of an old belief that lupin plants destroyed soil fertility, the name *Lupinus* was derived from the Latin word *lupus*, meaning a wolf (Everett, 1981). Phillips (1955) suggested that *Lupinus* was first used as a generic name by Tournefort (Institutious Rei Herbariae.1700). It was then established in the botanical literature by Linnaeus (sp.pl. 1753) (Phillips, 1955; Dunn and Gillette, 1966; Gladstones, 1974; Beltekey and Kovacs, 1984).

It is not intended to give an exhaustive review of the taxonomy and genetic relationships of *Lupinus* spp. A detailed account of the lupins of North America was presented by Phillips (1955). The complex genetic interrelationships of Alaskan lupins were clarified by Dunn (1965). A comprehensive taxonomic review of the lupins of Canada and Alaska was published by Dunn and Gillette (1966), while the taxonomy and description of the Mediterranean region and African species of *Lupinus* was discussed by Gladstones (1974). More recently, the lupins of the South America were reviewed and taxonomic studies reported by Plancheulo (1984). Dunn (1984) has considered the cytotaxonomy of the genus and reported the chromosome number of New World lupins along with their distribution.

The systematic framework of the genus *Lupinus* seems to be erected, primarily, from morphological data and ecogeographical studies. Many of the taxonomic reports concentrate primarily on studies of specimen collections of several herbariae (Phillips, 1955; Dunn, 1965; 1984; Dunn and Gillette, 1966; Dunn and Harmon, 1977; Plancheulo, 1984). Although these workers created a large measure of stability in the taxonomy of the

genus, there still exists a confusion in nomenclature and disagreement in delineating the taxonomy of lupins. The international working group for lupin descriptors (IBPGR, 1981) estimated some 300-400 species of lupin with several centres of diversity; however, over 1700 published names of lupins (varieties) have recently been estimated by Dunn (1984) and Beltekey and Kovacs (1984). The number of taxa warranting naming at the species or subspecific level was estimated to be about 500 (Dunn, 1984), with most of these species coming from South America.

2.1.2 Cytotaxonomic evidence

There is limited information on the chromosome numbers of *Lupinus* spp. Dunn and Gillette (1966), in their study of the lupins of Canada and Alaska, listed the chromosome numbers for 19 lupin species. With the exception of one species, which possesses n=24 and also n=48, they all have a haploid number of 24. The basic chromosome number suggested for the genus was six (Turner, 1957; cited by Dunn and Gillette, 1966; Dunn, 1984); in which case, most of the species would be octoploid (Dunn and Gillette, 1966).

Recently, a cytotaxonomic record of the genus was established by the study of herbarium specimens, from the Andes of South America to the Arctic of North America (Dunn, 1984). It was found that the genus is polyploid with chromosome counts of 36, 48, and 96, and one exception of 24. Therefore, based on a base count of x=6, the chromosome numbers in the genus should be present in sets of six, eight, and 16 replica or alleles (Dunn, 1984). This has led to the recognition of triploid, tetraploid, or octaploid gametes for New World lupin taxa (Dunn, 1984).

2.2 ORIGIN AND DISTRIBUTION OF *LUPINUS* SPP.

2.2.1 General

Lupins are believed to radiate from three main centres of diversity- the North Mediterranean region and north Africa, the Chile-Peru-Bolivia area of the South America, and the north western America (Phillips, 1955; Dunn and Gillette, 1966; Beleteky and

Kovacs, 1984). The Mediterranean and African lupins are annuals (Gladstones, 1974). The species in the American group are annuals, herbaceous perennial plants, semi-shrubs, and shrubby sorts. The latter are sometimes called tree lupins. Within the North American group, perennial lupin species predominate (Dunn and Gillette, 1966).

There is insufficient evidence to identify the exact place and time of domestication of *Lupinus* spp. The oldest known seeds of *Lupinus digitatus* Forsk, dating from before 2000 B.C., were argued to be found in Egyptian Pharoahs' tombs by Schweirfurth (undated, in Beletekey and Kovacs, 1984). It is generally believed that lupin growing originated in Egypt (Gladstones, 1970; Beleteky and Kovacs, 1984). However, the Greek word for *Lupinus albus* L. was *thermos* and the other names for the plant, *termis* and *turmus* in Egyptian language and Arabic respectively, appear to be derived from the Greek name (Aguilera and Trier, 1978). This suggests that the first cultivation of lupins as a crop may have been in Greece (Aguilera and Trier, 1978). Moreover, the earliest reference to the use of lupins in human nutrition was by the Greek physician Hippocrates' writing around the fourth century B.C. (Aguilera and Trier, 1978; Beleteky and Kovacs, 1984).

Modern interest in lupin growing expanded after 1780, when King Fredrik II of Prussia had seed of *L. albus* imported from Italy and initiated experiments on lupin cultivation (Hanelt, 1960; cited by Gladstones, 1970; Aguilera and Trier, 1978; Beleteky and Kovacs, 1984). The literature suggests that up to the late 1920's, lupins were primarily regarded as green manure crop (Gladstones, 1970; Beletekey and Kovacs, 1984), although *L. angustifolius* L. had been used in 1859 for sheep feed and green manure in Suffolk, United Kingdom (Beleteky and Kovacs, 1984). It has been speculated that the introduction of annual lupins to New Zealand may have been specifically for use as green manure (Gladstones, 1970; Beleteky and Kovacs, 1984). High alkaloid content (Gladstones, 1970; Aguilera and Trier, 1978; Beleteky and Kovacs, 1984), seed shattering, and hard seed (Gladstones, 1970) in the original lupin varieties were the major problems that limited the use of annual lupins until the late 1920's.

Two of the Mediterranean lupins freed from their toxic alkaloids in the late 1920s and 1930s by the German researcher, R. von Sengbusch, who isolated low alkaloid

(sweet) strains of *L. luteus* L. and *L. angustifolius* (Sengbusch, 1931; 1938; cited by Gladstones, 1970; Garside, 1975; Aguilera and Trier, 1978; Beleteky and Kovacs, 1984). Since then many sweet varieties of the *L. luteus, L. angustifolius*, and *L. albus* have been isolated and released throughout the world (Europe particularly in the USSR and Poland, the USA, Australia, and South Africa). Further-more, Gladstones and Francis (1965) and Baer and Gross (1983) also claimed to have isolated sweet strains of *L. cosentinii* and *L. mutabilis* respectively. While the late 1920s and 1930s marked the era of modern breeding and development of sweet lupins as grain and forage crops, commercial lupin crops are still confined to three annual species: *L. albus, L. luteus*, and *L. angustifolius*; although *L. mutabilis*, and *L. cosentinii* may soon be fully domesticated (Gladstones, 1980). It appears that perennial lupin species have been neglected in the breeding of sweet varieties.

2.2.2. Russell lupin

In 1911, George Russell began sowing the seeds of many different species and kinds of lupins. By the 1920's he had achieved astonishing results (Anderson, 1950; Hadfield, 1960). In June 1937, Russell lupins were first exhibited in public (Anderson, 1950; Hadfield, 1960; Gorer, 1970) at a show held in London by the Royal Horticultural Society (Anderson, 1950; Hadfield, 1960). Due to the diversity of colourful flowers they produce, Russell lupins were used to delight many of the visitors who had gathered in England to celebrate the coronation of King George VI and Queen Elizabeth (Anderson, 1950). Since then Russell lupins have gained widespread horticultural recognition and seem well known and are cultivated as decorative plants throughout the world.

Although George Russell did not undertake deliberate pollination, *L. polyphyllus* Lindl. appears to be the major parent of the Russell lupin, with *L. arboreus* Sims. (Gladstones, 1958a; Gorer, 1970; Chittenden, 1974; Dunn, 1984) and *L. nootkatensis* Sims. (Gorer, 1970; Dunn, 1984) contributing morphological traits and colours. Other possible species included in the Russell lupin are obscure. By and large, there has been hybridisation and subsequent introgression observed wherever *L. polyphyllus* has come in contact with other lupins (Dunn, 1965). This may be the reason why the chief herbaceous perennial lupins, cultivated as horticultural varieties, are hybrids of *L. polyphyllus*.

There is little published information on the introduced perennial lupin species in New Zealand. *Lupinus arboreus* was introduced in about 1904 (Wendelken, 1974) and was used in the coastal sand dune stabilisation programme. It was regarded as a naturalised plant in New Zealand and has colonised shrubby communities on coastal sands and gravels, riverbeds and railway sidings, in many areas of Canterbury (Healy, 1969). It was also common in sandy places near the sea on Stewart Island (Wilson, 1982).

Although *L. polyphyllus* and the Russell lupin were introduced into New Zealand, opinions differ concerning their distribution. Healy (1969) observed *L. polyphyllus* in berm communities in riverbeds, and railway sidings of the Mackenzie country of the South Island. He suggested that some stands of *L. polyphyllus* may have been artificially established, as was the case between Burkes Pass and Lake Tekapo (D.Scott, pers. com.). Mrs Connie Scott is believed to have introduced the multicoloured lupin seed into the Mackenzie country in 1952. However, the plants which have become naturalised in several regions of the South Island (Mackenzie basin, Fiordland, Lake Te Anau) are Russell lupins (Lambrechtsen, 1986; Horn and Hill, 1987). According to the latter authors, Russell lupins have escaped from gardens and invaded river banks, stream beds and road verges in the South Island regions. This agrees with Dunn (1984), who reported that the lupins commonly shown in New Zealand are the multicoloured Russell hybrid gone wild. Despite this, Lambrechtsen (1986) reported that *L. polyphyllus* has been used in revegetation programmes in New Zealand on moist, sandy or shingly seepage sites and shingly or rubbly scree slopes up to about 1000 metres. However, the small supply of *L. polyphyllus* seed limits large scale planting in New Zealand (Lambrechtsen, 1986).

Lupinus polyphyllus is an indigenous component of the flora of moist habitats in North America (Dunn, 1965; Dunn, 1984; Dunn and Gillette, 1966; Dunn and Harmon, 1977). It has a natural range extending from the San Jancinto Mountains of Southern California to British Columbia, Canada, and north to Alaska (Dunn, 1965; 1984; Dunn and Gillette, 1966; Dunn and Harmon, 1977). The species commonly occurs on sea shores, river banks, creekbeds and meadow, in areas of high rainfall, cool nights and cold winters (Dunn, 1965; Dunn and Gillette, 1966). The plant favours loose sandy or shingly soils which are moderately acid or slightly alkaline, and is not suited to tight, densely

compacted soils (Lambrechtsen, 1986). However, the plant has been introduced and cultivated in many countries of the world. Published information on the cultivated area and distribution of *L. polyphyllus* around the globe is scarce. A 1984 world report on lupins (Bellido, 1984) indicated that the species has been introduced and was cultivated widely in the USSR. It is planted as a fallow plant preceding wheat and rye in winter, and is used as a forage and green manure (Bellido, 1984). It was also introduced and cultivated in the southwest of Argentina where it was recently reported as 'wild' (Plancheulo, 1984).

Lupinus arboreus, commonly known as tree lupin, is indigenous to California and has a maritime habitat (Dunn, 1965; 1984). Because of its golden-yellow flowers, tree lupin has been deliberately introduced to many places. These include its long range dispersal along the coastal strand of Chile, Peru, England, the South Pacific Islands, and lake margins in Argentina (Dunn, 1984). The species hybridises readily with *L. polyphyllus* and other lupin species (Dunn, 1984).

Lupinus nootkatensis also has a maritime habitat and is indigenous to coastal areas of Alaska and British Columbia (Dunn, 1965). The species has been introduced into Newfoundland, Nova Scotia and Scotland (Dunn, 1965).

2.3 MORPHOLOGY OF THE RUSSELL LUPIN

There is little information on the morphological characteristics of the Russell lupins. Being composed of intraspecific hybrids it may not be possible to get Russell lupins to breed true from seed. The plant is a herbaceous perennial, which dies back to stout crown each winter (Horn and Hill, 1982; 1987; Wilson, 1982). The following description of the morphology of Russell lupin is from Horn and Hill (1982; 1987) unless otherwise stated. At maturity it grows to 1.5 m. It has very short stems which are usually inconspicuous during its vegetative growth period. The plant has a palmate leaf arrangement, the leaflets being quite hairless above and sparsely hairy on their undersides. The leaves arise from a rosette at the base with 15-30 cm long petioles. It has very long leaves, each with 9-16 pointed, lanceolate to oblanceolate leaflets of 5-15 cm long. The flowers, in an elongated terminal raceme up to 60 cm long, are individually up to 2 cm

long. The flowers have highly variable colours ranging from red, blue, cream, pink, orange and yellow to various combinations and shades of these colours (Gorer, 1970; Wilson, 1982; Horn and Hill, 1987). The plant produces wooly and hairy pods, 2.5 to 4.0 cm long, containing about 6 seeds. The seeds are brown to black, glossy and are about 0.3-0.5 cm long. Pods turn black and explode when dry (Wilson, 1982), making seed collection very difficult (D.Scott, pers. com.).

2.4 RESEARCH AND DEVELOPMENT ON THE PERENNIAL SPECIES OF LUPIN IN NEW ZEALAND

The role of tree lupin (*L. arboreus*) in New Zealand forestry has been under examination for almost 25 years (Sandberg and Gadgil, 1984). The significance of its contribution to the stabilisation of coastal sand dunes (Wendelken, 1974), and its contribution of biologically fixed nitrogen to young forest ecosystems on sand dunes (Gadgil, 1971a; 1971b; 1971c; 1976; 1979; Sprent and Silvester, 1973; Mead and Gadgil, 1978), have been acknowledged. It was demonstrated that most of the nitrogen demand of exotic forest development, such as *Pinus radiata* on an artificially stabilised coastal sand dune, could be met by the symbiotic nitrogen fixation of the tree lupin used in the initial stabilisation procedure (Silvester *et al.*, 1979).

The potential of legumes for the rehabilitation of levelled gold dredge tailings in the Taramakau River valley, New Zealand, was studied by Fitzgerald (1980). It was shown that Russell lupin and tree lupin were the only legumes to establish and grow readily without added fertilisers (Fitzgerald, 1980). This was confirmed by Davis (1981a; 1981b), who reported the ability of the Russell lupins to establish and grow under low soil phosphate conditions. In Davis' (1981b) pot and glasshouse studies which used an infertile acid soil and a high country yellow-brown sub-soil, Russell lupin was the only species among *Lupinus, Lotus, Trifolium, Lotononis, and Stylosanthes* spp. which was capable of growth with no applied phosphorus. Russell lupin was also the highest yielding species at low levels of applied phosphate (Davis, 1981b).

These results suggested that the strongly developed tap root and the greater seed reserves of the Russell lupin contribute to its greater P uptake and greater growth in low

available P environments (Davis, 1981a; 1981b). Further evidence of the Russell lupin's ability to establish at low temperatures and grow with minimal phosphate inputs was obtained at Lake Tekapo (Scott and Covacevich, 1987). On this high country grassland site, dominated by *Festuca novae-zelandiae* (fescue tussock) and *Pilosella officinarum* (mouse-ear hawkweed), Russell lupin was the only leguminous species that made any contribution to dry matter yield by the second spring in the absence of fertiliser. By the fourth spring, Russell lupin was one of the most successful species at annual rates of 50 to 250 kg of superphosphate ha^{-1}.

In a preliminary study at Lincoln College, the dry matter (DM) yield of alternative shrub legume species was compared with conventional pasture species (McKendry, 1987). In a high fertility Templeton silt loam site, 557 g DM m^{-2} was recorded from 130 days old Russell lupins. This was estimted by visual scoring and subsequent correlation (85.1%) of mean scores with reference harvested plants. Of this total production, 81% (453 g) was produced from plant parts which were less than or equal to 3 mm in diameter. This fraction of the plant was assumed to be willingly and readily consumed by stock. The edible yield of Russell lupin was comparable to the DM yield of 192 days old lucerne (442 g m^{-2}) and white clover (449 g m^{-2}) plants established from seed at the same site.

2.5 SOME FACTORS AFFECTING GERMINATION AND SEEDLING EMERGENCE OF HERBAGE LEGUMES

There is little published information on the agronomy and physiology of Russell lupin. To emphasize principles involved in the germination and emergence of herbage legumes, this section reviews work from other herbage legume species.

2.5.1 Hardseededness and acid scarification

There are two mechanisms which can prevent the germination of crop seeds, even if environmental conditions favour germination. These are hardseededness and dormancy. Hard seeds are defined as those seeds which do not imbibe water and fail to germinate at the end of a prescribed test period (Quinlivan, 1971; Khan, 1977; Rolston, 1978). Hard seed is common in the Leguminosae family (Quinlivan, 1971; Rolston, 1978) and occurs

in all three sub-families: the Papilionoideae; the Mimosoideae; and the Caesalpinoideae (Quinlivan, 1971).

The concept and theories of hardseededness which have attracted attention through the years were discussed by Khan (1977). Khan (1977) expressed the view that the release of seed dormancy is related to hormones initiating selective physical and metabolic changes at the molecular level. However, the seed coat and its structures have been implicated as physical barriers to restrict germination (Brant *et al.*, 1971; Rolston, 1978; Maguire, 1980). Many studies have shown that the physical barrier effects of the seed coat could be related to the exclusion of water, gases, or solutes (Khan, 1977; Rolston, 1978; Maguire, 1980) or light (Khan, 1977). Impermeability in legume seed is ascribed to the cuticle or the macrosclereid layer, also known as the palisade layer or the malpighian layer (Brant, *et al.*, 1971). However, this was challenged by Ballard (1973), who claimed that attempts to improve hardseededness, at least in *Trifolium* spp., should generally be via the strophiole rather than the testa. Yet, further work with *Medicago, Stylosanthes, and Trifolium* indicated that the strophiole and the general testa should be regarded as an integral system as the strophiole was found to be conductive without being acted upon directly (Ballard, 1976). A physical restriction, that restrains expansion and growth of the embryo, has also been suggested to be involved with the physical barriers of the seed coat (Khan, 1977; Rolston, 1978; Maguire, 1980; Duran and Tortosa, 1985).

Hardseededness in legume plants is genetically controlled (Quinlivan, 1971; Rolston, 1978), but its expression can be strongly influenced by environmental factors (Porter, 1949; Gladstones, 1958b; Quinlivan, 1971; Rolston, 1978). Relative humidity and temperature affect the degree of hardseededness retained in legumes prior to seed harvest. Leguminous plants, grown at sites with low relative humidity and high temperatures during seed development and maturation, tend to have high levels of hard seed. Furthermore, permeability in the West Australian blue lupin was found to be governed by seed moisture content (Gladstones, 1958b). Generally, permeability decreased with decreased seed moisture. This emphasizes the importance of post harvest environmental factors, especially storage conditions, for seed germination even after harvesting healthy, mature seeds. The possibility of breeding or selecting for a level of hard seed appropriate to a particular environment was suggested by Quinlivan (1971).

The ecological and agricultural significance of water impermeability of the seed coat of hard seed, in annual and perennial crops, was extensively reviewed by Rolston (1978). In perennial crops, hard seed may be considered as a potential reserve of the species, to fill empty patches or to help in re-establishing the species following unfavourable conditions. However, non-uniformity of plants and appearance of volunteers from buried seed for years following cropping with hardseeded varieties are some of the potential agronomic problems associated with hard seeds. If water impermeable seeds are to be used, a method is required to render planted seed germinable.

Various treatments that induce hard seeds to germinate have been known for many years. Early methods of seed treatment were reviewed by Porter (1949) and Quinlivan (1971) and more recently by Rolston (1978) and Maguire (1980). Artificial methods used to raise the percentage, or to shorten the period required to give optimum germination, can be classified as either 'wet' or 'dry' seed coat treatments. 'Wet' treatments include soaking in hot water, acids, organic solvents, adding oxidising agents, freezing, and the use of gases. 'Dry' treatments comprise heating, percussion, temperature fluctuation, micro-wave energy, nicking or chipping, and machine scarification.

Concentrated sulphuric acid (H_2SO_4) and mechanical scarification have been widely used with considerable successes on many species. Horn and Hill (1974) increased total germination by soaking seeds of *Lupinus cosentinii* in concentrated sulphuric acid (36N) for 4.0 to 7.0 hours. The 77% - 97% total germination of the acid treatments was not significantly different from the total germination of the hand scarified control (Horn and Hill, 1974).

The use of concentrated sulphuric acid on broom (*Cytisus scoparius* L.) seed for 2.0 to 5.5 hours and hand scarification (chipping) increased total germination from 2.5% to 75 - 88% (Wan Mohamed, 1981). Three to five and a half hours of soaking in 36N H_2SO_4 were required to produce 75% or more normal seedlings. While the number of abnormal seedlings tended to increase with increased immersion time in acid, hand scarified broom seed produced the highest percentage (29%) of abnormal seedlings (Wan Mohamed, 1981).

Liu, *et al.* (1981) reported a study to evaluate scarification treatments to promote seed germination in three woody legume species. In their study 120 or 150 minutes immersion in concentrated sulphuric acid increased germination of Kentucky coffeetree (*Gymnocladus dioicus* (L.)C.Koch) seeds to 90-95% compared with 4% in the untreated control. Likewise, 60, 90, or 120 minutes in concentrated sulphuric acid gave a 97-98% germination of honeylocust (*Gleditsia triacanthos* var. inermis L.) compared with 32% in the untreated control.

Voon (1986) compared different scarification treatments using concentrated sulphuric acid, hot water, mechanical scarification, chipping, and alternating temperatures. He found that soaking in hot water and alternating temperatures were least effective in overcoming hard seed in tagasaste (*Chamaecytisus palmensis* Christ) seed. Soaking tagasaste seeds for two hours in concentrated sulphuric acid gave 75% normal seedlings (% of seeds sown). It is possible that the scarification method required and the different time requirements for immersion in concentrated H_2SO_4 may be explained by species differences. Further, climatic conditions during seed maturation and the conditions under which seeds were collected, prepared and stored may also influence the degree of scarification required among seed lots within a species.

Despite the reported acid immersion experiments, information on the mode of action of concentrated H_2SO_4 is scarce. Exceptions are studies designed to examine the action of concentrated sulphuric acid on the seed surface of three woody legumes (Liu *et al.*, 1981) and on charlock (Duran and Tortosa, 1985), using a scanning electron microscope. They reported that lumens of the macrosclereid cells on the seed surfaces of Kentucky coffeetree, honeylocust, and redbud (*Cercis canadensis* L.) were exposed after acid treatment, permitting the imbibition of water. Germination of charlock seeds did not occur when the affected part involved the radicle or the embryonic axis. However, seedling emergence was accompanied by various degrees of abnormality only when parts of the cotyledons had been damaged. Duran and Tortosa (1985) concluded that the main action of concentrated H_2SO_4 on the seeds of charlock was the rapid dehydration of the seed coat cells which seems to allow the passage of oxygen to the embryo, rather than its strong oxidising or hydrolytic effects. Work with *L. angustifolius* and *L. arboreus* also

indicated that immersion in concentrated sulphuric acid ruptured the strophiole (G.D. Hill Pers. Com.).

2.5.2 Sowing depth

The sowing depth of pasture species is a managerial factor that helps to determine the availability of soil water to the seed and the ambient soil temperature. Usually, deeper sowing (2-5 cm) provides more available water and smaller daily fluctuations in temperature than sowing on or near the surface. Available soil water and soil temperature can be modified by plant cover (Janson and White, 1971; Voon, 1986). The optimum sowing depth for different species will also depend on seed size and seed food reserves.

Using small seeded herbaceous legumes, Peiffer *et al.* (1972) reported 80 % or higher emergence of crownvetch (*Coronilla varia*), lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*) seeds from depths of 1.3, 1.9, or 2.5 cm, but reduced emergence at 3.8 cm. In a glasshouse, where moisture was not limiting, higher emergence of broom seeds was recorded from 1-3 cm sowings compared to sowing at deeper than 3 cm (Williams, 1981; Wan Mohamed, 1981) or surface sowing (Wan mohamed, 1981). Significantly lower seedling emergence of broom was reported from 5 cm compared with 3 cm. However, at both 3 and 5 cm depth, the emergence rate of broom in fine soil was faster than in coarse soil (Williams, 1981). This may have been due to differences in the moisture retention characteristics and physical resistance of these soils. No broom seeds emerged from 10 cm sowing (Williams, 1981).

In field studies, higher and more rapid emergence was reported from overdrilled lucerne (Janson and White, 1971) and 1 to 3 cm sowing of tagasaste seed (Voon, 1986), compared with broadcast or surface sown seed respectively. Emergence of broadcast seed has been shown to depend on rainfall and plant cover (Cullen, 1969; Dowling *et al.*, 1971; Janson and White, 1971), which emphasises the importance of water relations in the soil surrounding the seed.

2.5.3 Temperature

Temperature is one of the most important factors in the external environment which controls the rate and duration of seed germination, seedling emergence, and subsequent plant growth.

There is general agreement that the germination rate of different herbage legumes slows as temperature moves from the optimum. Further-more, the lower the temperature the longer the time required to germination (McWilliam *et al.*, 1970; Young *et al.*, 1970; Silsbury *et al.*, 1984; Hampton *et al.*, 1987). However, controlled environment studies have shown that the final germination and emergence of lucerne (McWilliam *et al.*, 1970; Young *et al.*, 1970; Hampton *et al.*, 1987), white clover (*Trifolium repens*) (Mcwilliam *et al.*, 1970; Hampton *et al.*, 1987), and red clover (Hampton *et al.*, 1987) were insensitive to constant temperature over the range of 5 to 20 ^{o}C. Although cultivars of subterranean clover (*Trifolium subterraneum*) varied sightly in their germination response to constant temperature (Hampton *et al.*, 1987), final germination and emergence of subterranean clover was not affected over the range 10 to 20 ^{o}C (McWilliam, *et al.*, 1970; Young *et al.*, 1970; Silsbury, *et al.*, 1984; Hampton, *et al.*, 1987). All of these workers found that while low temperatures (_5 ^{o}C) did not inhibit the germination of some subterranean clover cultivars, moderately high temperatures, i.e. >25 ^{o}C, reduced germination and emergence in most of the legumes investigated.

Over two seasons, using oversown lucerne and white clover on unimproved sites at Tara hills in the Mackenzie country, Musgrave (1977) showed that maximum establishment of these species occurred when the mean 10 cm soil temperature following oversowing was in the range 3-7 ^{o}C. At both earlier and later sowing times, there were lower and higher temperatures respectively, and seedling establishment of oversown lucerne and white clover was considerably lower than the maximum.

Generally, the minimum temperature required for germination is less than the temperature required for emergence and subsequent seedling growth. Thus it seems likely that it is only when the temperature falls substantially below 5 ^{o}C that low temperature becomes a major factor limiting legume establishment.

For successful emergence and field establishment of herbage legumes in hill and high country of New Zealand, it is essential to know and define the minimum temperature requirement of alternative potential pasture species.

2.6 CHARACTERIZATION OF PLANT SPECIES FOR USE IN PASTURES ON ACID SOILS OF LOW P STATUS.

With the continuous growth of world population, crop and livestock production has to be increased to meet growing food demand. Success will be achieved partly through searching for plants that will grow under the existing climatic and edaphic conditions, or by improving plants through plant breeding to take better advantage of the climate or improved soil fertility. The basis of this task is primarily plant collection and exploration. Consideration of environmentally homologous regions in plant collection and introduction areas is usually seen as an index of the likelihood that new plant species will be adapted to the region where they are required (Creech, 1970; Burt *et al.*, 1976; 1979; Williams *et al.*, 1976; Burt and Forde, 1986; Keoghan, 1986; Tyler *et al.*, 1987a). However, incidence of plant diseases and pests may differ from one country or region to another. Therefore, evaluation of the potential of new germplasm is necessary during plant introduction. Without evaluation there can be no effective utilisation of new plant resources. As any evaluation of germplasm is resource demanding, the formulation of appropriate objectives for an evaluation programme is a necessary prerequisite.

There are two main reasons for formulation of the objectives in the initial stage of an evaluation programme when plant introduction is considered. Firstly, because of site differences in soil, climate, vegetation, agricultural practices and pest and disease incidence the long-term objectives of one country's or region's plant introduction programme will differ from another. Secondly, because of existing inherent genetic variability of plant species, plant characteristics differ from one genus and/or species to another. It is therefore important to clearly define the type of plants being sought and the areas that are likely to give the best opportunity for pasture development before any collection, testing and evaluation of germplasm for use in pasture is made.

New Zealand hill country is characterised by soils which are acid and of inherently low soil fertility, a climate with low winter temperatures, and seasonal soil moisture deficits (Sinclair and McIntosh, 1983; Chapman and MacFarlane, 1985; Scott *et al.*, 1985). Dry matter production is low from resident vegetation. Until recently, the dry matter production and feed quality of hill country pastures had been improved by the introduction of legumes following the correction of soil nutrient deficiencies (White, 1984; 1989; Chapman and MacFarlane, 1985; Scott *et al.*, 1985). In turn this has improved stock carrying capacities and animal performance. However, this approach may no longer be feasible given the current high development costs together with low returns from meat production. To-date, total fertiliser applied by air has declined from the peak of 1.26 million tonnes in 1980 to only 0.27 million in 1987 (White, 1989). This has led farmers and scientists to consider alternative legume species for hill and high country pastures, which are better adapted to soil and climate than the lowland grasses and clovers presently being introduced.

Desirable attributes of alternative pasture legumes for such environments are:-

- A tolerance of acid soil conditions including high Al concentrations;
- The ability to absorb P which is not readily available, and other soil nutrients, by rooting morphology and/or rooting depth;
- The ability to nodulate effectively in acidic, low nutrient soils and improve soil N status;
- A tolerance of temperature extremes, especially low winter temperatures and freezing;
- A tolerance of drought by having deep roots, dormancy etc.;
- The ability to withstand grazing or browsing;
- The ability to respond to additions of fertiliser when it is economic to apply it;
- The ability to produce a reasonable yield of quality forage acceptable to livestock.

It is important that screening of any alternative legume pasture plant for the high country should include several of the above criteria. Breeding of pasture plants adapted to the environment is a comparatively new approach compared with changing the environment by large additions of fertiliser and lime. Because of this recent involvement in the field of pasture breeding, the characterization of pasture plants is still in the

investigative stage. However, the effort made by Commonwealth Scientific and Industrial Research Organisation (CSIRO), Tropical Pasture Research Division, Welsh Plant Breeding Station (WPBS), and others is commendable.

The next section will discuss points that should be considered during plant introduction, initial evaluation and screening of new plant species for use in pastures.

2.6.1 The need for plant introduction and sources of plant material

Collection of plant material for use in pastures is the first step in the sequence of events leading to the utilization of new germplasm in breeding programmes or pastoral situations. Plant collections are assembled for a variety of reasons. The most important reasons for collecting forage plant species are for preservation of endangered species, for exchange purposes, for the improvement of native pastures low in quality and carrying capacity, or for further improvement of an already improved pasture. Genetic material for use in plant introduction may be obtained from plant collection and exploration mission, from seed exchange (Williams *et al.*, 1976; Strickland *et al.*, 1980; Jones *et al.*, 1984; Tyler *et al.*, 1987a), via donations from institutes and seed merchants (Tyler *et al.*, 1987a), or by purchase from commercial sources.

Germplasm collection for use in pastures may be obtained from many sources. These include plant material of wild relatives, plants cultivated elsewhere, or in the experimental stage at local or international research centres, and/or commercial seeds. This difference in the state of germplasm collection necessitates different approaches to a plant evaluation programme. The evaluation process for commercial seeds or experimental lines seems different from that for wild species. Information on the growth characteristics, feeding value, and behaviour under grazing in another environment can be obtained for commercial seed or experimental lines. The fact that seed is available commercially means that initial seed increase programmes are not required, and that larger areas can be sown at more sites from importation of a large seed lot. Therefore, if adapted plant species can be obtained directly from commercial sources, a plant introduction programme can thus proceed more rapidly than is the case for wild species, which need to be subjected to all stages of evaluation programmes (Shaw *et al.*, 1976; Jones *et al.*,

1984). However, because of site differences in soil, climate, vegetation, agricultural practices, and disease and pest incidence, along with the inherent genetic variability of plant species, the need for the evaluation of their potential in both commercial and wild plants, for their use in pastures, is apparent.

2.6.2 Characterization and preliminary evaluation

Characterization and preliminary evaluation of observable characters are given priority during initial studies of germplasm collections (IBPGR, 1981; Frankel, 1986; Tyler, *et al.*, 1987b).

> "Observable or strongly expressed characters can be readily identified in single plants or their progenies, although this may require special conditions such as the presence of a pathogen or a specific - usually extreme-environment, but not a multiplicity of diverse environments. They are simply, or if polygenic, strongly inherited and can be readily selected for in hybrid generations ..... evaluation carried out on germplasm collections is generally confined to observable characters" (Frankel, 1986).

Characterization and preliminary evaluation of pasture species often combines glasshouse and field evaluation (Burt and Williams, 1979; Cameron and McIvor, 1980; Rhodes, 1987; Tyler *et al.*, 1987b). Glasshouse studies usually concentrate on evaluating seedling characters as a guide to establishment potential in the field. However, seed quality, which is largely determined by environmental conditions at harvest, is likely to affect the value of screening for seedling characters. Tyler *et al.* (1987b) suggested that it is necessary to assume uniform conditions at seed multiplication for seedling characters to be usefully studied. They claimed that glasshouse evaluation of seedling characters, dry weight and morphology, are the most informative for forecasting the establishment potential of introduced forage plants in the field.

The characterization and preliminary evaluation of germplasm collections often involves the measurement of morphological characters of spaced plants growing in the field (Williams, 1983; Tyler *et al.*, 1987b; Rhodes, 1987). The following methods of preliminary screening of temperate forage grasses (Tyler *et al.*, 1987b), and characterization of white clover (Rhodes, 1987), by the Welsh Plant Breeding Station have recently been described.

After a short recovery from cutting in the glasshouse, individual grass seedlings are taken from the glasshouse to harden up outside. These are transplanted into rows of spaced plants in the field. It is possible at this stage to make morphological measurements or estimates on single plants for characters such as plant height, length and width of the flag leaf, height of the inflorescence, habit, disease at inflorescence emergence and number of inflorescences.

The study of many vegetative and flowering features such as leaf size, stolon characteristics and flowering date along with observation on pest and disease damage have been recommended for characterization of white clover introductions (Rhodes, 1987). These should be measured both on young pot-grown white clover plants in the glasshouse as well as on spaced white clover plants grown in the field.

Although considerable amounts of information on the value of an accession to a breeding programme may be obtained from spaced plants, single plant evaluation has limited value (Rhodes, 1987; Tyler *et al.*, 1987b). Because of inadequate interplant competition, seasonal yield of single plants has no consistent relationship with sward yield. Also, because of lack of information on sward characteristics, it is not possible to estimate persistence as a major sward characteristic. Mini-plots are recommended when seed or land are not limiting, as yield estimates are likely to correlate better with swards and observations on sward characteristics are possible. However, if possible, large plots are preferable (Rhodes, 1987; Tyler *et al.*, 1987b).

2.6.3 Desirable attributes of plant species for use in pastures

The agronomic traits required from pasture plant species have been discussed by Williams *et al.* (1976), Shaw *et al.* (1976), and Jones *et al.* (1984). The desirable characteristics of plant species for use in pastures can be considered under three headings.

(a) Ability to grow and persist

Plant species must grow and persist under existing conditions of soil and climate, whether by persistence of individual plants, or the natural spread through seed, stolons, or

rhizomes. Depending upon the area of future pasture development, plants must be drought tolerant and/or resistant to cold and frost. Moreover, resistance to pests and diseases is very important.

Pasture plants must be tolerant of heavy grazing, including periodic extreme grazing pressure, and should be able to set seed over an extended period while being heavily grazed. In short, plant species for use in pasture must be capable of naturalising in existing environmental and pastoral conditions.

(b) Ability to produce a high yield of quality forage

The total dry matter yield of plants is very important. It is particularly important to have a high leaf to stem ratio. The plant must be of reasonable palatability and free from toxic substances. Further more, it is desirable in legumes that they nodulate readily.

Although tolerance to low soil fertility and acid soils is important in reducing fertiliser and lime requirements, pasture species should be able to respond to improved soil fertility.

(c) Ease of propagation

Successful plants for use in pasture should establish easily. This may be either by seed or vegetative material. It should also be easy to collect such material. Although vegetative propagation is possible in most species, establishment by seed is often the only feasible way for large-scale development of pastoral agriculture.

Since the important attributes of pasture plants vary with environment and with the objective of the pasture improvement programme, there is no set order of importance for the agronomic characteristics listed above. For example, good drought tolerance or avoidance is vital for plants being selected for areas with a long dry season, but is of minor importance in areas with reliable year-round rainfall. Also, there is no perfect species. For example, *Lotus pedunculatus* cv. Maku, is considered to be more suitable for improving the fertility of high country soils of New Zealand than the clovers which are commonly used (Lowther, 1977; Nordmeyer and Davis, 1977; Scott and Lowther, 1980;

Davis, 1981a; 1981b; Lucas *et al.*, 1981; Scott and Mills, 1981), but has limitation in that it is often slow to establish (Lucas *et al*, 1981; Scott and Mills, 1981) and slow to recover after grazing (Sheath, 1980a; 1980b; 1981).

Potentially dangerous and toxic plants should be removed during the preliminary evaluation stage. Moreover, plants that are excessively coarse, or badly damaged by insects or diseases, can also be eliminated from further testing. Although the main objective of initial evaluation is to select the best plants for further work and to discard plants of little or no value as quickly as possible, the period of preliminary study should enable similar accessions to be grouped together so that only one or a few from each group needs to be carried into the later evaluation stages. This grouping can be helped by the use of a multivariate statistics.

2.6.4 **The use of multivariate statistics**: application to plant introduction

In the search for better plants, a large number of plant accessions are being collected, introduced and evaluated in many areas throughout the world. Usually, preliminary evaluation relies on the analysis of a complex mass of agronomic and morphological data from large populations of accessions. Several statistical options are available to help in this. The use of univariate statistics is widespread in many research programmes and is applicable where one variate is measured. If a number of correlated measurements are made, it is usually unwise to carry out a separate analysis of variance on the individual variates. Rather, the use of several multivariate statistical techniques are possible.

It is not intended to give an exhaustive review of the multivariate techniques used for plant introduction purposes. However, the most commonly used numerical analysis of variation patterns in plant evaluation data during spaced-plant trials seem to include classification (Burt and Williams, 1979; Williams, 1983; Bishop, *et al.*, 1988) and ordination techniques (Burt and Williams, 1979; Burt, *et al.* 1979; Williams, 1983; Bishop, *et al.* 1988; Thomson, 1974). Thomson (1974) in Britain examined winter hardiness components in 24 perennial ryegrass varieties using canonical variate analysis. Principal component analysis and factor analysis were used to study fruit characteristics of

11 species of vertebrate-dispersed plants of the Iberian Peninsula (Herrera, 1987) in Spain. Classification and ordination (Principal coordinate analysis and minimum spanning tree) techniques were applied in the evaluation, classification, and description study of a collection of 57 *Stylosanthes* accessions (Burt and Williams, 1979) and 316 accessions of the legume genus *Aeschynomene* (Bishop, *et al.*, 1988) in CSIRO, Australia. Fernandez de la Reguera, *et al.* (1988) applied a multiple-set canonical analysis to investigate the existence of natural hybrids between two *Pinus* spp. sharing the same geographical area.

Classification deals with the subdivision of a large population (of the order of several hundreds) into manageable groups. It is considered as the simplest and most effective starting point in explaining the structure of plant evaluation data (Williams, 1983). But, classification postulates that the population under study is discontinuous, and discontinuity exists. However, there will be considerable continuous variation. Methods for the study of continuous variation are collectively known as ordination techniques (Williams, 1983).

Multivariate analysis of variance (MANOVA) may be used if the individuals belong to an experimental design grid (Mardia *et al.*, 1979; Chatfield and Collins, 1980). In such cases MANOVA deals with all the variates simultaneously, or as a vector, taking into account not only their separate variances, but also their covariances in all possible pairs which are collected in a matrix of sums of squares and products (Chatfield and Collins, 1980). Multivariate analysis of variance tests the null hypothesis that there are no differences in class means. If there are differences in the class means, then the null hypothesis is rejected. It is then necessary to study in detail the discrepancies between the null hypothesis and the data and decide the required number of dimensions. If necessary, there are multivariate statistical options available to compare the sample means, in a reduced dimensionality than that of the number of measured variables. These include canonical variate analysis (CVA) and principal component analysis (PCA) (Pearce, 1969; Mardia *et al*, 1979; Chatfield and Collins, 1980; SAS Institute, 1985)..

If the individuals belong to a number of distinct but internally homogenous groups (Seal, 1966), CVA will give axes which optimally separate the groups. Canonical variate analysis assumes a number of groups of individuals on which a set of variates has been

measured. The method aims to find linear combinations which maximize the between-group variation relative to the within-group variation (Mardia *et al*, 1979; Chatfield and Collins, 1980). (Further discussion of this statistical technique is presented in Chapter 4). However, if the array has no given structure like the case of ungrouped data, PCA is useful for studying the dependence of a set of variables (Mardia *et al*, 1979; Chatfield and Collins, 1980). The purpose of this statistical technique is to derive a small number of linear combinations of a set of variables called principal components. Hopefully, this small number of principal components will retain as much of the information contained in the original variables as possible.

Any pattern in adaptation and extrapolation to other environments may also be discerned by using multivariate statistical data analysis. However, interpretation of genetic resource data from preliminary evaluation and characterization under glasshouse studies, and from spaced plants in the field, is relevant to the requirements of breeders or agronomists working in similar environments (Tyler *et al.*, 1987b; Rhodes, 1987). Further evaluation is necessary to enable meaningful characterization and to make more reliable extrapolation to other environments.

CHAPTER THREE

SEEDLING EMERGENCE STUDIES OF RUSSELL LUPIN

3.1 INTRODUCTION

The establishment of herbage legumes has two distinctive phases: (i) seedling emergence, and (ii) seedling establishment. Seedling emergence is defined here as being when the cotyledons completely appear above the surface of the soil or the appearance of the radicle at the soil surface. Provided nutrients are non-limiting, the proportion of hard seed, soil moisture, sowing depth, and ambient temperature are all factors which are important and influence the rate of seedling emergence and the effective establishment of herbage legumes.

Preliminary laboratory tests which were designed to measure the germination of freshly harvested Russell lupin seed gave 41-54% germination after 10-15 days. The germination of Russell lupin seed stored at room temperature for 8 months was also 54% or less after 10 days. The low germination was probably due to the impermeability of the seed coat to water, i.e. a hard seed. If successful seedling establishment of the Russell lupin is to be achieved there is a need to improve the germination of hard seeds.

As reviewed in Chapter 2, a number of seed scarification techniques have been tested and are widely used with considerable successes on many legume species. A preliminary test indicated that with hot water treatment, Russell lupin seed was killed at 100 ^{o}C. Horn and Hill (1974) found similar results in *Lupinus cosentinii*. Nevertheless, preliminary results suggested that scarification by chipping or with concentrated sulphuric acid (36N) could improve the germination of Russell lupin seed. Once the potential of these treatments was evident, it was decided to investigate the effect of scarification by chipping and with sulphuric acid on the germination and emergence of Russell lupin seed.

Sowing depth determines the availability of soil moisture to the seed as well as affecting the ambient soil temperature. Surface sowing of legumes is generally less reliable than overdrilling or sub-surface sowings (Janson and White, 1971; Williams,

1981; Wan Mohamed, 1981; Voon, 1986), although cost and the risk of soil erosion are generally lower. The reduced reliability of surface sowing is the result of the less favourable temperature and moisture regime for seed germination at the soil surface than those experienced by buried seed. The highest emergence of lucerne (Janson and White, 1971; Peiffer *et al.*, 1972), red clover and crownvetch (Peiffer *et al.*, 1972), broom (Williams, 1981; Wan Mohamed, 1981) and tagasaste (Voon, 1986) was obtained by sowing at 1-3 cm. There is no published information for Russell lupin on their response to sowing depth. It was essential therefore to investigate the rate and final total emergence of Russell lupin in response to sowing depth.

Although, most temperate herbage legumes germinate over the range of 5 to 20 ^{o}C (McWilliam *et al.*, 1970; Silsbury, *et al.*, 1984; Young *et al.*, 1970; Hampton *et al.*, 1987), low temperatures (<5 ^{o}C) are recorded regularly in New Zealand hill (shady faces) and high country sites in winter (Musgrave, 1980). It is also well documented that the germination rate of different legume species decreases as temperatures move away from the optimum, although the optimum germination and/or emergence temperature varies among species. There is no published information on the effect of temperature on the germination and emergence of Russell lupin. Thus, for successful emergence and establishment in the hill and high country environment of New Zealand, the minimum temperature requirement for germination of Russell lupin must be defined.

This chapter reports a series of laboratory and glasshouse experiments which were designed to:-

(i) investigate different scarification methods to obtain optimum seed germination and emergence of normal Russell lupin seedlings;

(ii) examine the effect of depth of sowing on seedling emergence and seedling vigour of Russell lupin;

(iii) observe the effect of temperature on the rate and total emergence of Russell lupin.

3.2 THE EFFECT OF CONCENTRATED SULPHURIC ACID (36N) ON THE GERMINATION AND EMERGENCE OF RUSSELL LUPIN SEED

3.2.1 MATERIALS AND METHODS

3.2.1.1 EXPERIMENT ONE: Laboratory germination study

i. Experimental material and treatments

Seeds of Russell lupin were collected from naturalised plants in a road side stand in the Mackenzie basin, between Burkes Pass and Lake Tekapo, Canterbury, New Zealand (Latitude 44^{o} 33$^{'}$S, Longitude 170^{o} 33$^{'}$E). Pods with mature seeds were collected on 22 January 1988. Pods were dried and threshed. Shrivelled seeds were discarded and seed stored at room temperature until required.

The seed scarification treatments tested were chipping, and immersion in concentrated sulphuric acid (36N) for 0, 15, 30, 45, 60, 90, and 120 minutes.

Chipping:- The testa of each seed was nicked at the end opposite to the hilum with nail clippers.

Concentrated sulphuric acid treatments:- Seed samples were placed in perforated pots and dipped in concentrated sulphuric acid (36N). Following the method suggested by Hartman and Kester (1968), two volumes of acid to one volume of seed were used. After 15, 30, 45, 60, 90, and 120 minutes of immersion in the acid, duplicate samples of 200 seeds were removed from the acid. Following thorough washing in running water for 15 minutes, the seed was air-dried at room temperature in the shade. Untreated seed and the chipped seed were washed and dried similarly.

For each treatment, 200 seeds were placed on moist germination discs in four petri dishes (i.e. 50 seeds/dish). These were placed in a seed germinator at 20 ^{o}C. The petri dishes were inspected daily and watered with distilled water as required.

ii. Data collection

The total number of seeds germinated after 10 days was counted. A seed was considered to have germinated when it showed a radicle about the length of the seed. There was no separation of normal and abnormal seedlings.

iii. Data analysis

Germination results were taken as a percentage of total seeds sown. Percent emergence values were arc sin transformedto insure against non-homogeneity of variance.

The data was analysed with the Statistical Analysis System (SAS) programme. As the transformed data and non-transformed gave similar results, results for non-transformed data are presented for ease of discussion and interpretation.

3.2.1.2 EXPERIMENT TWO: Seedling emergence from sand

i. Experimental material, layout and treatments

Russell lupin seed for this study was collected, prepared, and stored in the same way as in Experiment 1. The seed scarification treatments were also similar except that immersion periods in acid were from 0 to 3 hours at 15 minutes intervals.

Samples of 100 seeds per treatment were sown at about 1 cm depth in a 45 x 40 x 6 cm wooden box filled with unsterilised sand. A 3 x 4 cm spacing between seeds was maintained during sowing. The experiment was a randomised block design with three replicates in a glasshouse. The boxes were inspected daily and watered as required.

ii. Data collection

The number of emerged seedlings in each treatment was recorded daily for the first 10 days after sowing and finally on day 15. In most instances the complete appearance of the cotyledons above ground level was considered as seedling emergence. Following the

International Rules of Seed Testing Association (I.S.T.A., 1966), emerged seedlings were sorted into normal or abnormal seedlings. Common abnormalities included intact testa, twisted roots, and the failure of the cotyledons to free themselves from the testa (Plate 3.1).

iii. Data analysis

The emergence of total seedlings (normal + abnormal), normal seedlings, or abnormal seedlings were recorded as a percentage of total seeds sown. Percent emergence values were arc sin transformed to insure against non-homogeneity of variance.

The rate of emergence was calculated for each scarification treatment using the method of Maguire (1962).

$$\text{Emergence rate} = \frac{\text{Percentage of newly emerged seedling at day } \mathbf{n}}{\mathbf{n}}$$

where **n** was the number of days from sowing.

The speed of germination of each seed treatment was also derived by estimating the days to onset of emergence and the time taken to obtain 50% emergence.

Analysis of variance on the data was performed using the Statistical Analysis System (SAS) programme. As in Experiment 1, the transformed data and non-transformed data gave similar results, therefore, again, results for non-transformed data are presented.

Curves for the percentage emergence of total seedlings, normal, or abnormal seedlings in response to the time of immersion in acid were derived by regression using Minitab. Quadratic and cubic equations were used to fit the line to emergence values whenever it was significant ($p \leq 0.05$), otherwise the linear equation was utilized.

Plate 3.1 Abnormal (1 & 2) and normal (3) Russell lupin seedlings from the method of scarification study in sand:

(1) Abnormal seedlings - predominantly from chipping;

(2) Abnormal seedlings - predominantly from longer immersion periods in concentrated sulphuric acid; and

(3) Normal seedlings at different growth stages.

3.2.2 RESULTS

3.2.2.1 Experiment one: Laboratory germination

Results of this experiment are shown in Figure 3.1. Over all treatments there was a highly significant difference in germination response. The maximum germination of 98% was obtained by chipping, whereas the lowest, at 35%, was the control. Soaking seeds for 90 and 120 minutes in sulphuric acid both gave 88% germination, but this was not significantly different from the percentage germination obtained by acid scarification for 30, 45, or 60 minutes.

3.2.2.2 Experiment two: Seedling emergence in sand

i. Total seedlings emerged

At day six, there was no significant difference in the initial seedling emergence among seed treatments (Figure 3.2). Highly significant differences ($p \leq 0.001$) among scarification treatments were obtained between 7 and 15 days after sowing.

The highest total emergence of seedlings of 92%, after 15 days, was obtained by chipping. However, this was not significantly different ($p > 0.05$) from the 30, 45, 60, or 75 minute acid immersion treatments (83-89% emergence). Immersion periods of more than 60 minutes in the acid reduced the final total seedling emergence (Figures 3.2, 3.3). The relationship between time of immersion in acid and emergence of total seedlings, after 15 days in sand, was cubic ($Y = 44.1 + 1.66X - 0.0185X^2 + 0.000056X^3$, $R^2=79.3\%$) (Figure 3.3). The lowest emergence at 40%, after 15 days, was from untreated seeds.

ii. Emergence of abnormal seedlings

The percentage of abnormal seedlings emerged is shown in Figure 3.4. The highest proportion of abnormal seedlings at 36% was from chipped seeds. This was not significantly different ($p > 0.05$) from the 32% abnormal seedlings produced by 180

minutes in acid. The proportion of abnormal seedlings after 15 days in sand, increased linearly ($Y = 2.93 + 0.152X$, R^2=75.6%) with increased time of immersion in acid (Figure 3.3). Control seeds produced less than 1% abnormal seedlings (Figures 3.3, 3.4).

While intact testa was the most common abnormality observed from chipped seeds, the appearance of twisted roots in Russell lupin seedlings was the most common abnormality recorded for plants which had been immersed for long periods in concentrated sulphuric acid (Plate 3.1).

iii. Emergence of normal seedlings

The emergence of normal seedlings is shown in Figure 3.5. Acid immersion for 30, 45, or 60 minutes produced more than 70% normal seedlings after 15 days. The lowest normal seedlings emergence was from 180 minutes in acid. However, this was not significantly different ($p>0.05$) from the control, and immersion in acid for 150 or 165 minutes. A cubic relationship, ($Y = 46.4 + 1.23X - 0.0152X^2 + 0.000045X^3$, R^2 = 75.3%), was found between time of immersion in acid and the proportion of normal seedling emergence after 15 days in sand (Figure 3.3).

iv. Emergence rate of normal seedlings

The rate of emergence of normal seedlings is shown in Table 3.1. The first sign of seedling emergence was 5 to 6 days after sowing. The rate of normal seedling emergence was significantly improved by scarification with concentrated sulphuric acid for 30, 45, or 60 minutes compared with untreated seed, chipped seed, or seed which had been longer in the acid. Seed which had been in acid for >150 minutes, and untreated seed never gave a 50% seedling emergence and were consistently lower than the other treatments. The fastest rate of seedling emergence was from seed which had been in acid for 30 or 45 minutes.

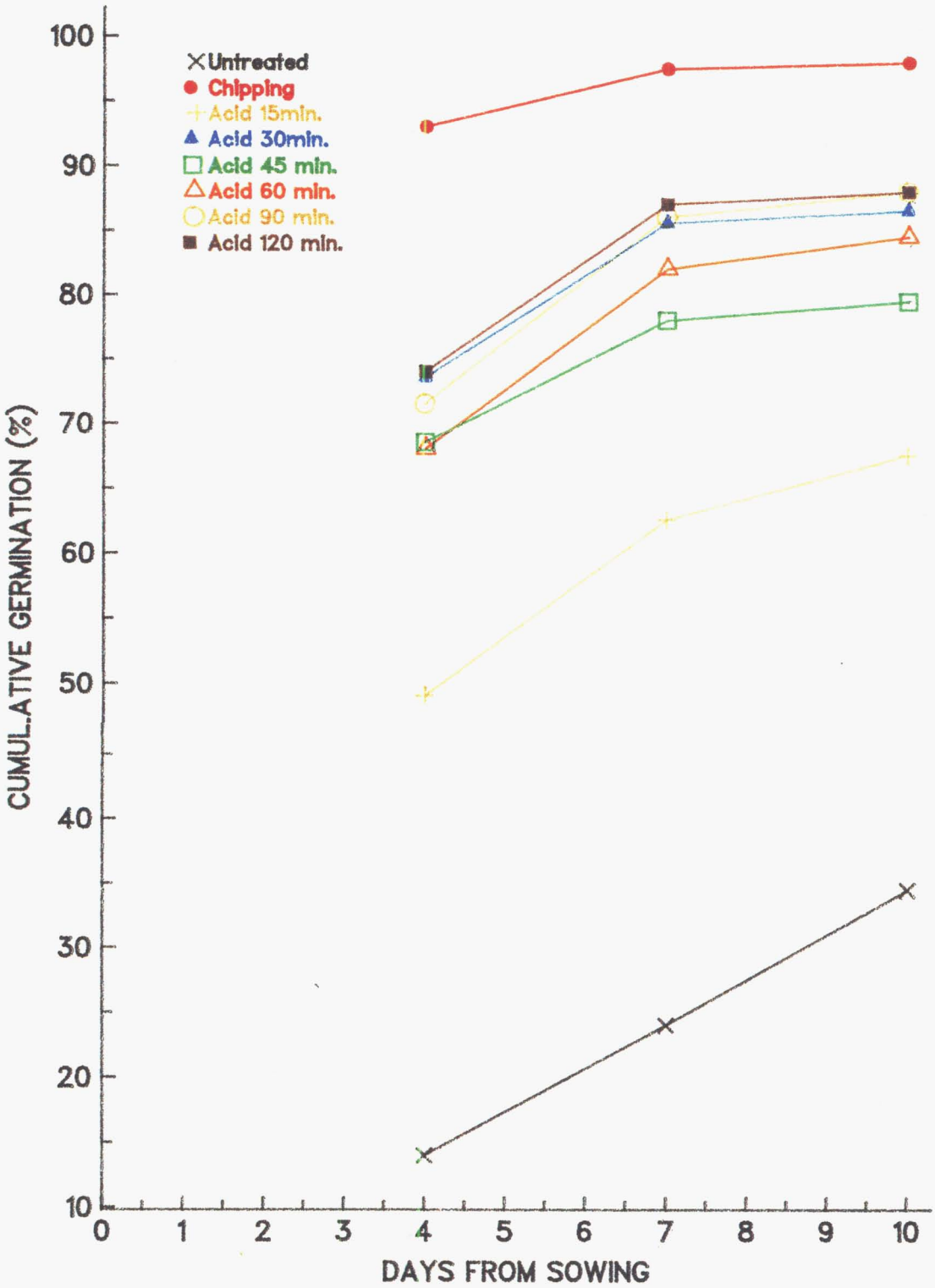


Figure 3.1. The effect of method of scarification on the laboratory germination of Russell lupin.

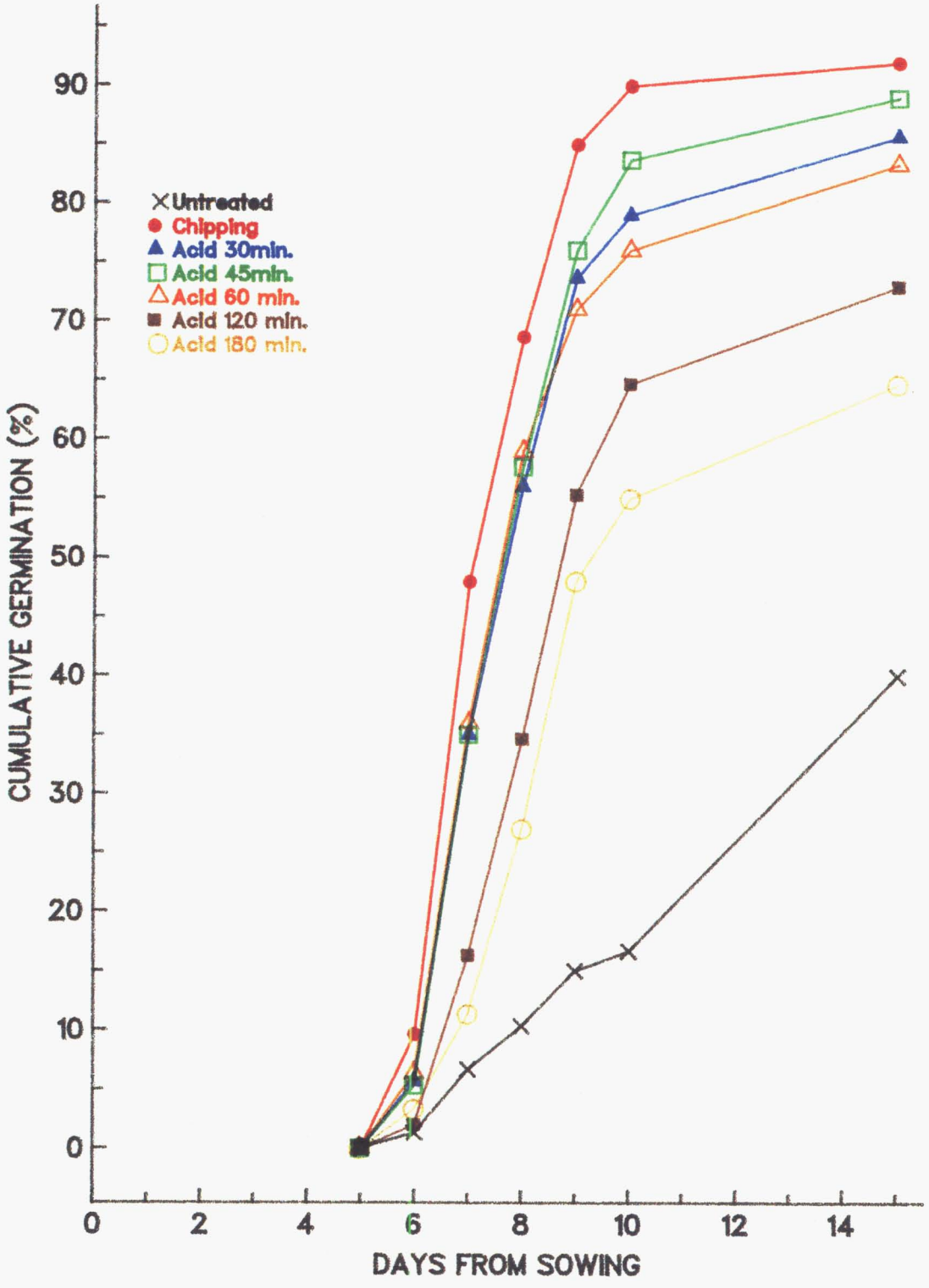


Figure 3.2 The effect of the scarification method on total seedling emergence of Russell lupin in sand.
(Full results in Appendix 1, Table 2).

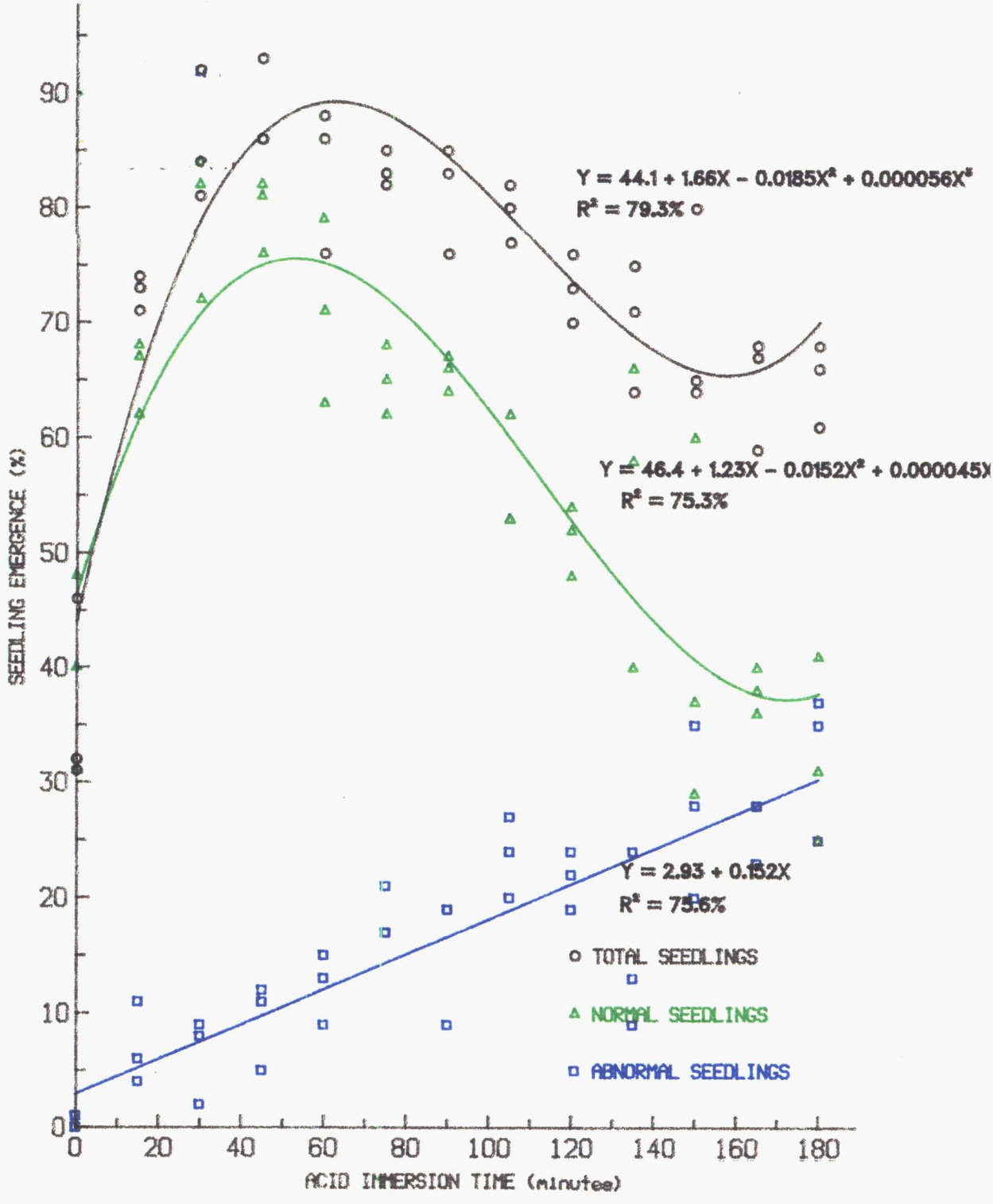


Figure 3.3 The relationship between period of immersion in concentrated sulphuric acid (36N) and the emergence of total, normal, and abnormal seedlings of Russell lupin at 15 days after sowing, in glasshouse.

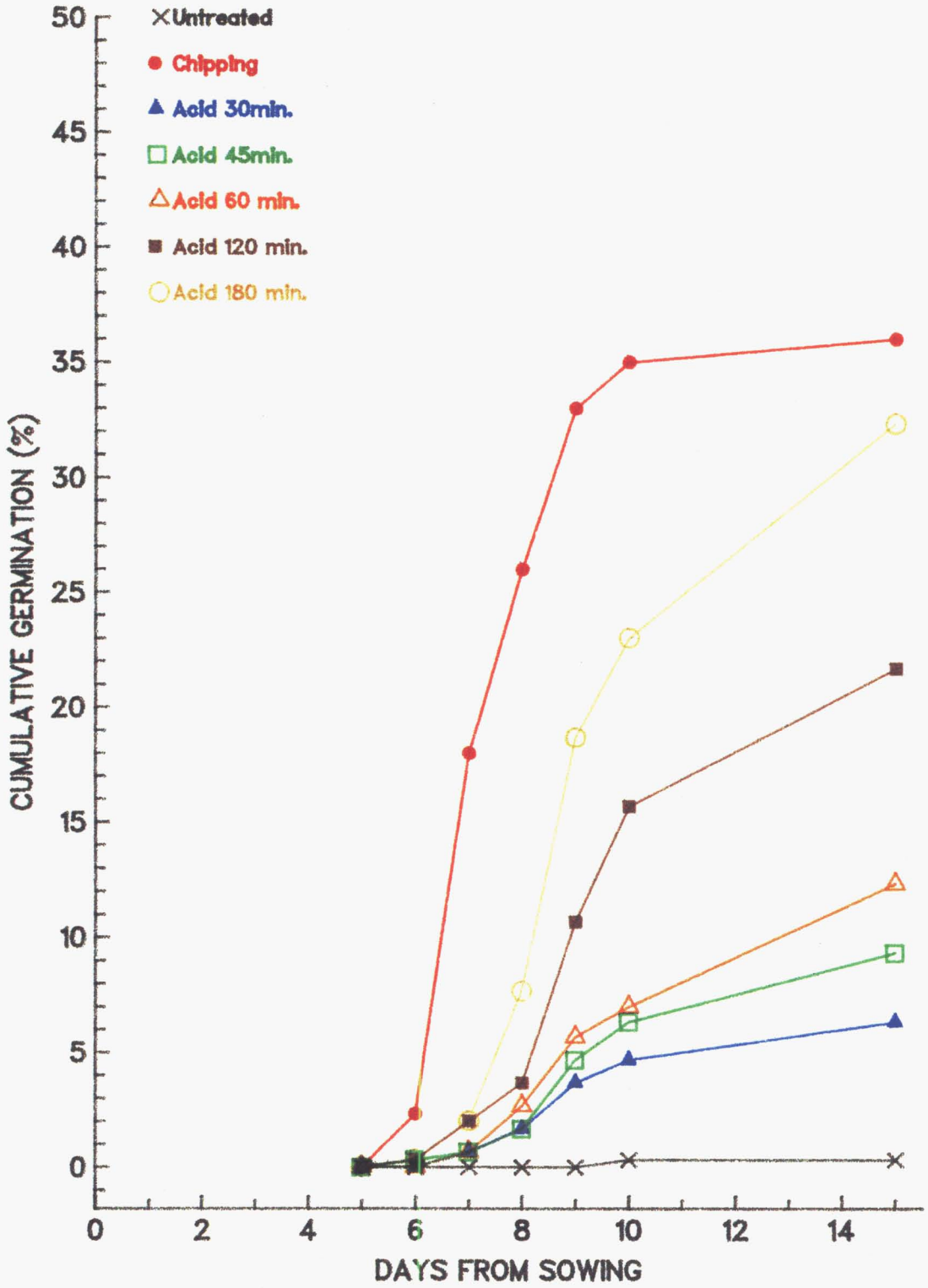


Figure 3.4 The effect of the method of scarification on abnormal seedling emergence of Russell lupin in sand.
(Full results in Appendix 1, Table 3).

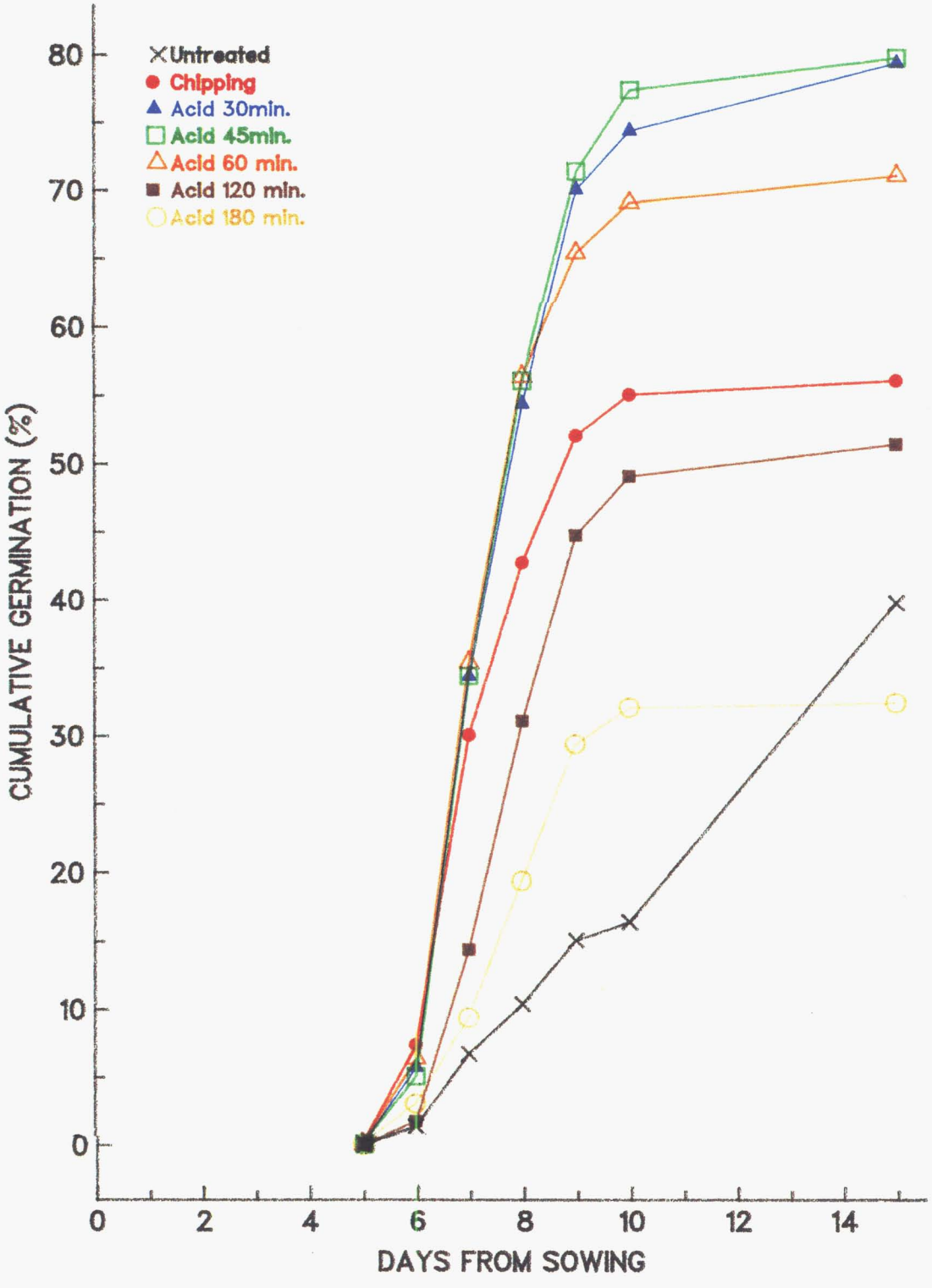


Figure 3.5 The effect of the method of scarification on normal seedling emergence of Russell lupin in sand.
(Full results in Appendix1, Table 4).

Table 3.1. The effect of scarification method on days to onset of emergence, days to 50% emergence, and the emergence rate of normal seedlings of Russell lupin grown in sand in a glasshouse.

Immersion in acid (minutes)	Days to onset of emergence	Days to 50% emergence[1]	Emergence rate
0	5.5	-	3.7
15	5.2	8.7	8.0
30	5.2	7.5	10.2
45	5.2	7.6	10.0
60	5.2	7.7	9.3
75	5.2	8.4	8.2
90	5.2	8.5	8.2
105	5.2	9.0	7.1
120	5.2	10.0	6.2
135	5.2	9.8	6.6
150	5.2	-	5.0
165	5.2	-	4.7
180	5.2	-	4.7
Chipping	5.1	8.6	7.4
Significance	NA	NA	***
$LSD_{(0.05)}$	NA	NA	1.56
CV (%)	NA	NA	13.10

[1] - Final emergence percentages were less than 50%.

NA - Not Available.

3.3 THE EFFECT OF SOWING DEPTH ON THE EMERGENCE AND SEEDLING VIGOUR OF RUSSELL LUPIN

3.3.1 MATERIALS AND METHODS

i. Experimental material and treatments

Russell lupin seed for this study was collected, prepared, and stored in the same way as in the scarification study (Section 3.2).

Seed was scarified in concentrated sulphuric acid (36N) for 45 minutes as in the previous experiments (Section 3.2). Scarified seed was placed on moist germination discs in petri dishes. The petri dishes were placed in a seed germinator at 20 ^{o}C and were inspected daily and watered with distilled water as required during the four day germination period.

For each treatment, 100 pre-germinated seeds were sown at 3 x 4 cm spacing in 45 x 40 x 6 cm wooden boxes which had been partially filled with unsterilised sand. Seeds was then covered with sand to the required depth.

The experiment tested sowing depths of 0, 1, 2, 3, 4, and 5 cm below the soil surface. The design was a randomised complete block with three replicates. The boxes, were placed in a glasshouse, inspected daily and watered as required.

ii. Data collection

The number of emerged seedlings was recorded at day 4, 7, 10, and 15 after sowing, and then weekly until 57 days after sowing. The date of sowing of pre-germinated seeds was taken as day zero. A seed was considered to have emerged, either when the cotyledons were above the soil surface (for sub-surface sown seeds), or when the radicle penetrated the soil surface (for surface sown seeds).

The maximum and minimum glasshouse air temperature were recorded daily.

Seedling vigour was studied on randomly selected seedlings from each treatment. Twenty nine days after sowing, emerged seedlings in each treatment were thinned to leave 15 seedlings per box. This was done to minimise possible interplant competition caused by the large differences in the number of emerged seedlings among treatments. Seedlings which emerged after 29 days from sowing were counted and removed. However, because of the small number of emerged seedlings in the 4 and 5 cm sowings, observations of seedling vigour were confined to the 0, 1, 2, and 3 cm sowing depths.

Fifty seven days after sowing, the soil surface level of each seedling was marked with a permanent marker. The 15 seedlings in each treatment were dug up and the complete seedlings harvested from each box. As much root material as possible was recovered for each seedling. The excavated seedlings were immediately washed in tap water, and the length of the shoot and the root were measured. The plant material from each treatment was partitioned into shoot and root fractions and was placed in an oven drier. The oven dry weight of the shoot and root fractions was recorded.

The experiment started on 24 May 1988 was completed on 20 July 1988.

iii. Data analysis

The seedling emergence in each treatment was taken as the percent of the total seeds sown. The values were arc sin transformed.

The rate of emergence for each treatment was calculated using the method of Maguire (1962) as in Section 3.2. The speed of emergence at each sowing depth was also derived by estimating the days to the onset of emergence and the time taken to obtain 50% emergence.

Seedling growth parameters were analysed on a per seedling basis. The root:shoot ratio of each treatment was calculated by dividing the weight of the root by the weight of the shoot.

The data were analysed using the Statistical Analysis System (SAS) Programme. For ease of discussion and interpretation, emergence results are reported as percentages that were de-transformed back to the original units.

3.3.2 RESULTS

i. Air temperature of the glasshouse

The maximum and minimum air temperatures in the glasshouse during the experimental period (24/5/88 - 20/7/88) are shown in Figure 3.6. The daily minimum air temperature in the glasshouse ranged from 1.5 to 13.5 ^{o}C. The mean daily minimum air temperature was 7 ^{o}C. The daily maximum air temperature ranged from 12.5 to 28 ^{o}C. The mean daily maximum air temperature was 19 ^{o}C. The mean daily air temperature throughout the experiment was 13 ^{o}C.

ii. Emergence rate

The effect of sowing depth on the emergence of Russell lupin is shown in Figure 3.7 and Table 3.2. The first seedling emergence which was realised by the 0 and 1 cm depth, occurred at 6 to 7 days after sowing of the pre-germinated seed. The rate of seedling emergence was significantly improved in the 1 or 2 cm depth compared with sowing either at the surface or deeper than 3 cm. The latter treatments never achieved a 50% emergence and had consistently lower emergence than the 1 and 2 cm treatments (Figure 3.7). The fastest seedling emergence was from the 1 cm sowing.

iii. Final emergence

There were highly significant differences ($p \leq 0.001$) among the sowing depths (Figure 3.7). Fifty seven days after sowing, the highest seedling emergence at 92% was from the 1 cm sowing depth. The lowest emergence of 3% and 4% was from sowing at 5 and 4 cm depths respectively. Surface sowing only gave an emergence of 34%; and was similar to the seedling emergence from 3 cm (Figure 3.7).

iv. Seedling vigour

There was no significant difference ($p>0.05$) in shoot height among the treatments (Table 3.3). However, significant differences were recorded for all of the other seedling vigour measurements. The longest root length of 11.8 cm was from the 1 cm sowing; but this was not significantly different ($p>0.05$) from the 9.8 cm in the 2 cm sowing. Similarly, the highest seedling shoot and root DM, and root:shoot ratio were from the 1 cm sowing. However, seedling shoot and root DM yield and root to shoot ratio from the 1 and 2 cm treatments were not significantly different ($p>0.05$).

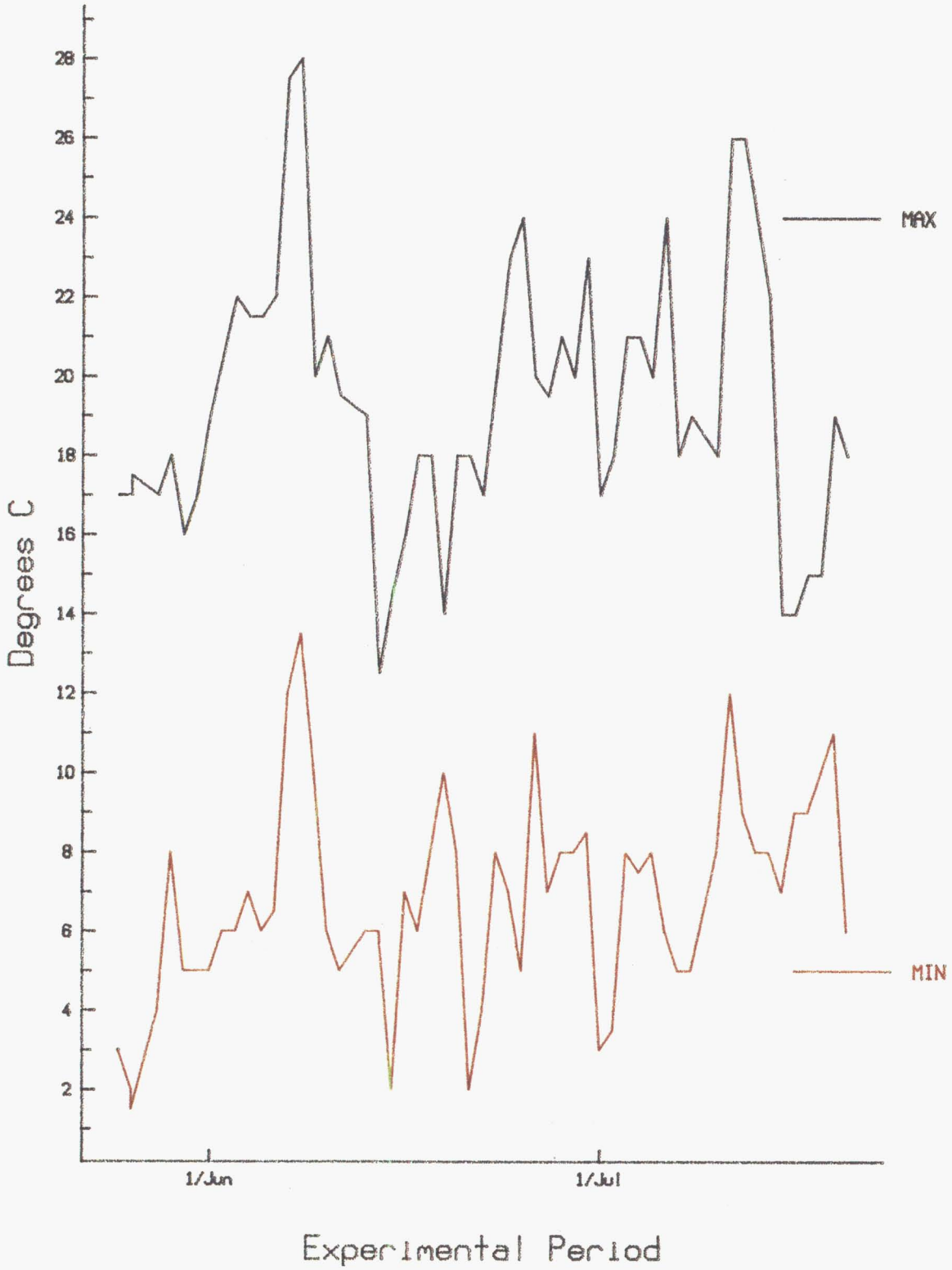


Figure 3.6 Daily maximum and minimum glasshouse temperature during the study of sowing depth effects on the emergence and seedling vigour of Russell lupin in sand.

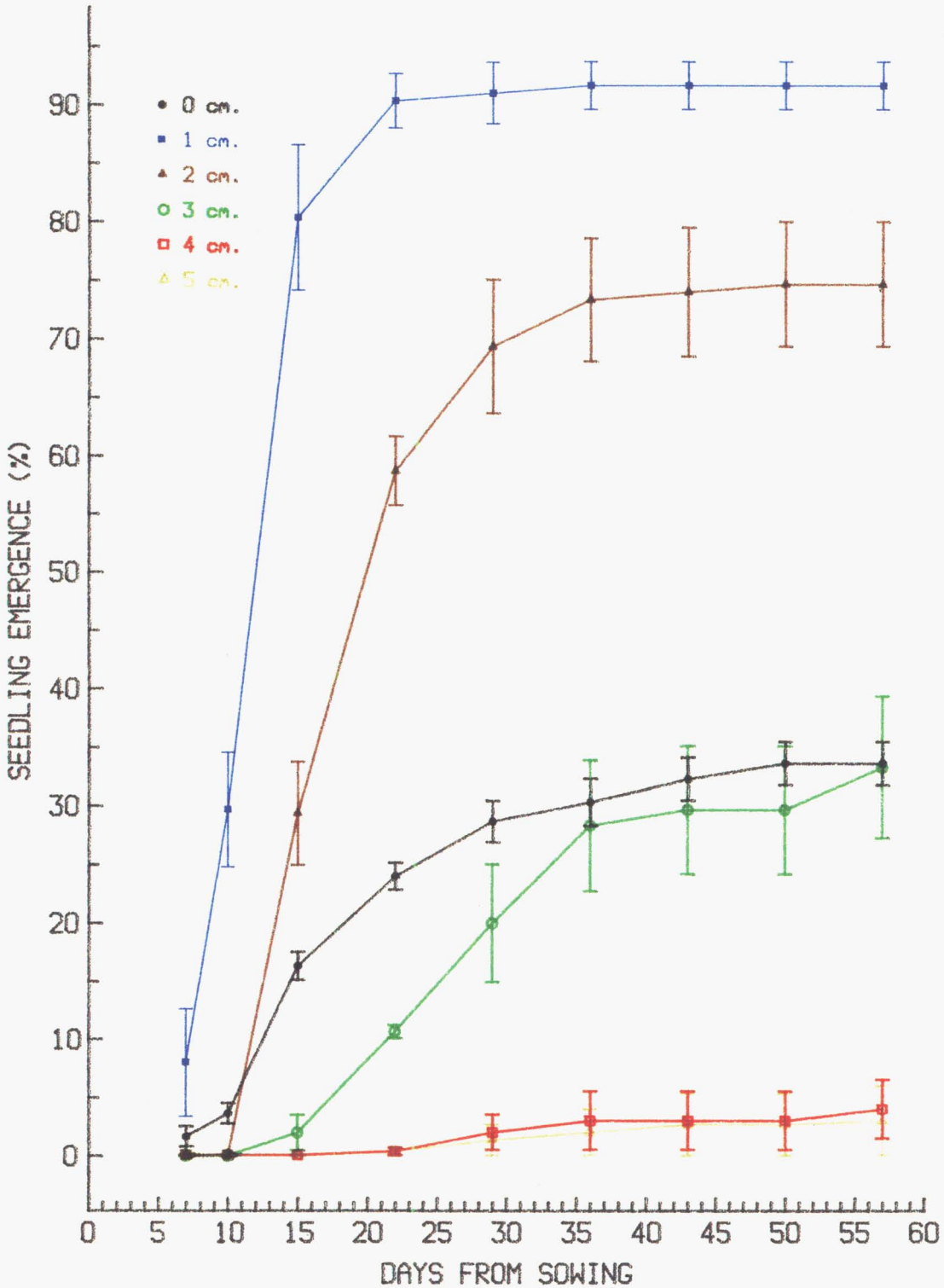


Figure 3.7 The effect of sowing depth on the emergence of Russell lupin seedlings in glasshouse. (Bars indicating ± SE of mean).

Table 3.2. The effect of sowing depth on days to onset of emergence, days to 50% emergence, and the emergence rate of Russell lupin grown in sand in a glasshouse.

Sowing depth (cm)	Days to onset of emergence	Days to 50% emergence[1]	Emergence rate
0	6.8	-	1.9
1	6.1	10.3	7.2
2	10.1	20.0	3.8
3	12.5	-	1.2
4	25.1	-	0.1
5	27.5	-	0.1
Significance	NA	NA	***
$LSD_{(0.05)}$	NA	NA	0.88
CV (%)	NA	NA	20.30

[1] - Final emergence percentages were less than 50%.

NA - Not Available

Table 3.3. The effect of sowing depth on the growth of Russell lupin seedlings after 57 days in a glasshouse.

Sowing Depth (cm)	Shoot Height (cm)	Root Length (cm)	Shoot DM (mg plant^{-1})	Root DM (mg plant^{-1})	Root to shoot Ratio
0	4.3	8.1	46.7	17.8	0.38
1	4.9	11.8	57.8	33.3	0.56
2	5.4	9.8	55.6	28.9	0.51
3	4.3	7.7	46.7	20.0	0.42
Significance	ns	*	***	***	***
LSD $_{(0.05)}$	1.64	2.68	4.96	7.36	0.07
CV (%)	17.40	14.30	4.80	14.70	10.30

Significance levels are represented by **ns = non-significant,**

*** = p≤0.05, ** = p≤0.01, and *** = p≤0.001**

3.4 OBSERVATION ON THE EFFECT OF TEMPERATURE ON THE EMERGENCE OF RUSSELL LUPIN

3.4.1 MATERIALS AND METHODS

i. Experimental material and treatments

Seed was obtained from the same source as in Section 3.2. and scarified in concentrated sulphuric acid (36N) for 45 minutes as in the previous experiments (Section 3.2, 3.3).

For each treatment, 100 scarified seeds were sown at about 1 cm depth in 30 x 25 x 6 cm plastic box filled with unsterilised sand. The experiment was conducted in temperature controlled cabinets at constant temperatures of 10, 15, 20, or 25 ^{o}C with 14 hours of light and 10 hours dark daily. Because of the lack of germination cabinets, the temperature treatments could not be replicated. Boxes were inspected daily and watered as required.

ii. Data collection

The number of seedlings emerged in each treatment was recorded daily for the first 10 days after sowing and then on days 12 and 15. The complete appearance of the cotyledons above ground level was considered as seedling emergence.

iii. Data analysis

The emergence of seedlings in each treatment was recorded as the percent of total seeds sown. Because of the absence of replication the data could not be statistically analysed. The rate of emergence was calculated for each temperature using the method of Maguire (1962). The speed of germination for each temperature treatment was also derived by estimating days to the onset of emergence and days to 50% emergence.

3.4.2 RESULTS

i. Emergence rate

The speed of emergence of Russell lupin in response to temperatures from 10 to 25 ^{o}C is shown in Table 3.4. The rate of seedling emergence increased with increasing temperature and the slowest rate of emergence was at 10 ^{o}C. Increasing the temperature from 10 to 25 ^{o}C also decreased the period from sowing to the onset of seedling emergence and the time to 50% emergence.

ii. Final emergence

There was only a slight difference in the final emergence for all treatments except at 25 ^{o}C, where the total emergence was considerably reduced (Figure 3.8). The highest final emergence of 92% was obtained at 20 ^{o}C. Emergence was reduced to 66% at 25 ^{o}C.

Table 3.4. The effect of temperature level on days to onset of emergence, days to 50% emergence, and the emergence rate of Russell lupin.

Temperature ^{o}C	Days to onset of emergence	Days to 50% emergence	Emergence rate
10	8.1	10.4	7.7
15	3.5	6.8	12.4
20	3.1	6.2	14.5
25	2.2	5.4	15.1

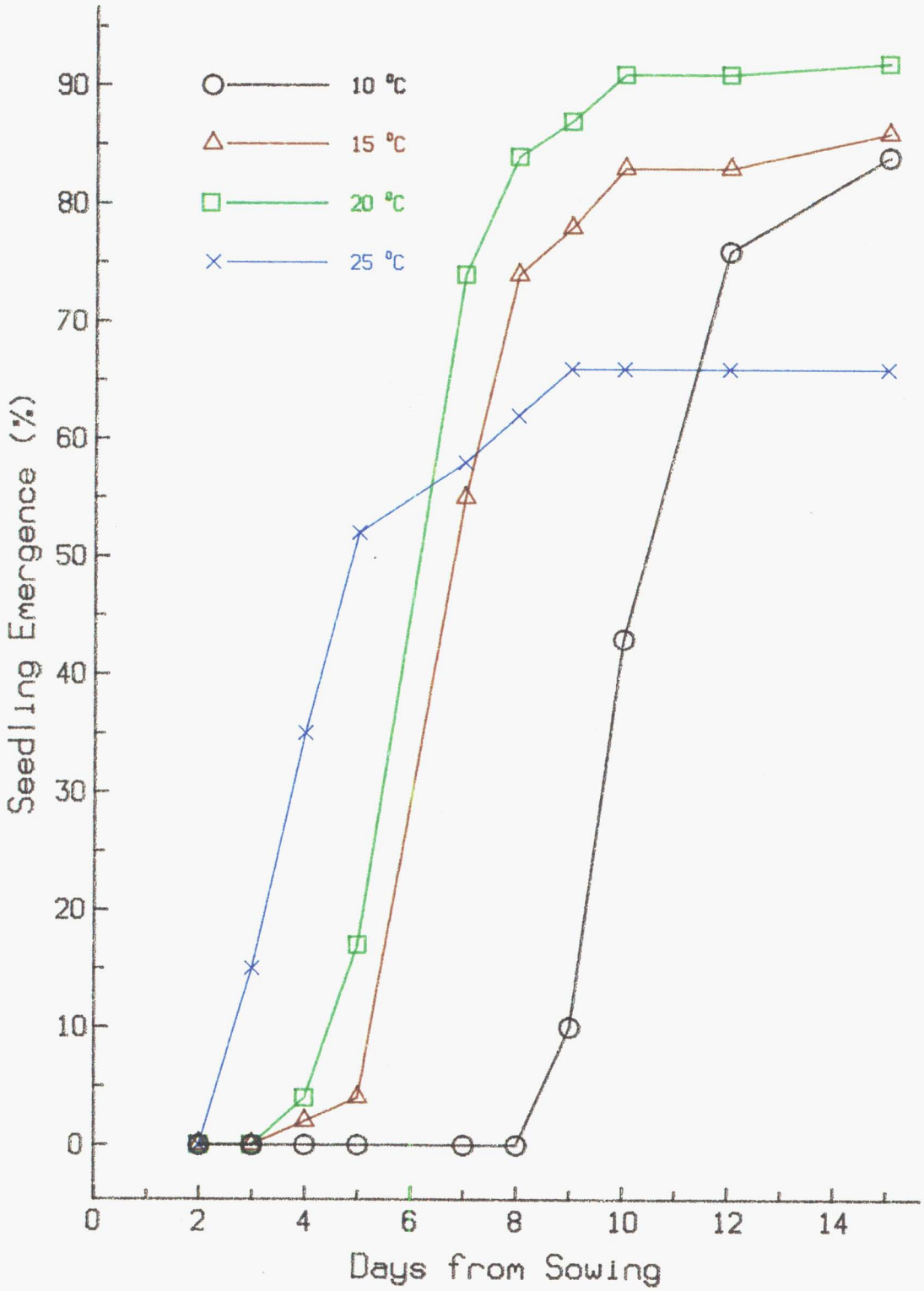


Figure 3.8 The effect of temperature on emergence of Russell lupin seed in sand

3.5 DISCUSSION

3.5.1 Scarification method

Experiment 1 (Section 3.2.2.1) and Experiment 2 (Section 3.2.2.2) suggest that the best germination and emergence of freshly harvested Russell lupin seed could be obtained by scarification by chipping or using sulphuric acid. However, as shown in Experiment 2 (Figure 3.4) a large number of abnormal seedlings (36%) were produced by chipping. The results from the chipping treatment were comparable to emergence from hand scarified seeds of *L. cosentinii* (Horn and Hill, 1974) and broom (Wan Mohamed, 1981). While Horn and Hill (1974) obtained a 96% emergence from hand scarified *L. cosentinii*, Wan Mohamed (1981) increased the emergence of broom from 2.5% in the control to 79.0% in seeds scarified by hand. In Wan Mohamed's (1981) work, chipping produced 29% abnormal broom seedlings after 50 days in a glasshouse. Although chipping improved the total, and the rate of germination, because of the abnormal seedlings, it was of a little value as mechanical scarification only produced 41% more normal seedlings than the control (Figure 3.5). This was significantly less than the emergence of normal seedlings from the best acid scarification treatment.

The response of Russell lupin seed to concentrated sulphuric acid confirmed earlier work by Horn and Hill (1974), Wan Mohamed (1981), Liu, *et al.*,(1981), and Voon (1986), who reported that scarification with concentrated sulphuric acid improved the germination of *L. cosentinii*, broom, three woody legume species (honeylocust, kentucky coffeetree, and redbud), and tagasaste seeds.

Although the final emergence of Russell lupin increased significantly with all immersion periods in sulphuric acid compared with the control (Figure 3.2), longer immersion periods reduced final seedling emergence (Figures 3.2, 3.3). There was a linear increase in the emergence of abnormal seedlings with increased acid immersion (Figure 3.3). Thus, emergence of more than 70% normal seedlings was only obtained from seeds scarified for 30, 45, or 60 minutes acid immersion (Figures 3.3, 3.5). These treatments gave from 80 to 100% more normal seedlings than the control. Although the form of abnormality in acid scarified broom seeds was not reported, the number of

abnormal seedlings increased to 10.5% as the period of acid immersion was increased from 1 to 5 hours (Wan Mohamed, 1981). At any one same period of immersion in acid, the number of abnormal seedlings in Russell lupin was always higher than in broom.

3.5.2 Effect of sowing depth

When grown in a glasshouse where moisture was not limiting, seedling emergence and seedling vigour of Russell lupin were affected by sowing depth.

Fifty seven days after sowing of pre-germinated seed, seed sown at 1 and 2 cm gave 170% and 120% respectively more emerged seedlings than seed sown at the soil surface or at 3 cm (Figure 3.7). Although seedling emergence from surface sowing was slightly faster than from 3 cm, these treatments gave a similar final emergence (Figure 3.7). Sowing Russell lupin seed at 4 or 5 cm below the soil surface gave a significantly lower ($P<0.001$) rate and final emergence than the other treatments.

The results of this study for surface sown and shallow sowings show that in spite of its larger seed size Russell lupin is similar to other herbage legumes and lends support to the findings of Janson and White (1971), Peiffer *et al.* (1972), Williams (1981), Wan Mohamed (1981), and Voon (1986). These studies reported that the highest emergence of lucerne (Janson and White, 1971, Peiffer *et al.*, 1972), red clover and crownvetch (Peiffer *et al.*, 1972), broom (Williams, 1981, Wan Mohamed, 1981), and tagasaste (Voon, 1986) was obtained from sowing at 1-3 cm compared with seed sown either at deeper than 3 cm or at the soil surface.

The low rates of emergence of this experiment may have been due to low air temperatures in the glasshouse (Figure 3.6), as the emergence of Russell lupin was slowed by low temperature (Section 3.4). Although it would have been soil temperature that had an influence on germination and emergence of surface and sub-surface sown seeds, there would have been a direct relationship between the glasshouse air temperature and the soil temperature. Observations of seed sown at 4 and 5 cm showed that many of the seeds had germinated but had failed to reach the soil surface. It is possible that deeply sown seeds

which failed to emerge died once their food reserve was exhausted and before they emerged.

Despite regular watering, only 34% of surface sown Russell lupin seed was established 57 days after sowing. This may have been due to drying of the soil surface between consecutive waterings which would have given poor seed/soil water contact, thereby limiting imbibition.

Although there was no significant difference in seedling shoot height; root length and oven dry weight of the shoot and the root significantly decreased ($p>0.05$) in seedlings which emerged from 3 cm depth and in the surface sown seedlings. Best seedling vigour was exhibited by seeds sown at 1 cm, but these were not significantly different ($p>0.05$) from the 2 cm sown seedlings.

These emergence and seedling vigour results suggest that the optimum sowing depth for Russell lupin is between 1-2 cm below the soil surface.

3.5.3 Effect of temperature

Temperature in the range of 10 to 25 ^{o}C markedly influenced both the speed and final emergence of Russell lupin.

The main effect of increased temperature was a decrease in the lag period. The lag phase was 8 days at 10 ^{o}C and this was decreased to 2 to 3 days at 20 to 25 ^{o}C (Table 3.4). Comparisons of the lag period and the time to 50% emergence clearly show that the speed of emergence was slowed by low temperatures. There was also some evidence of the influence of temperature on the emergence rate (Table 3.4), although differences among the 15, 20, and 25 ^{o}C treatments, for speed of emergence, were relatively small.

Total seedling emergence, 15 days after sowing, increased from 10 to 20 ^{o}C but decreased considerably at 25 ^{o}C. The evidence indicated that constant temperature in the range from 10 to 20 ^{o}C is not a limiting factor in total emergence of Russell lupin. The reduced emergence at the highest tested temperature suggested that Russell lupin is

sensitive to germination temperatures $\geq$ 25 ^{o}C. This study also demonstrated that the minimum germination and emergence temperature for Russell lupin is less than 10 ^{o}C.

Studies on the effects of temperature on seed germination and emergence have been conducted on lucerne (McWilliam *et al.*, 1970; Young *et al.*, 1970; Hampton *et al.*, 1987), white clover (McWilliam *et al.*, 1970; Hampton *et al.*, 1987), red clover (Hampton *et al.*, 1987), and subterranean clover (McWilliam *et al.*, 1970; Young *et al.*, 1970; Silsbury *et al.*, 1984; Hampton *et al.*, 1987). In most of these experiments, although germination rate fell as temperatures moved away from the optimum, final germination and emergence differed little over the range of 10 to 20 ^{o}C. Therefore, Russell lupin also seems to fall into this temperature/emergence response category.

3.6 CONCLUSIONS

Russell lupin seed collected in mid-summer had a high proportion of hard seed, and untreated seed only gave about a 50% germination in laboratory germination tests. However, the seed can be treated to break hardseededness. The experiments reported in this chapter show that the rate and final emergence of Russell lupin depended on the method of seed scarification, the depth of sowing, and germination temperature.

In glasshouse studies, it was shown that more than 75% emergence of normal seedlings could be obtained by:-

1. scarification by immersion in concentrated sulphuric acid (36N) for 30 to 45 minutes;
2. sowing at 1 to 2 cm below the soil surface; and
3. germinating at a temperature of 10 to 20 ^{o}C.

The results suggest that Russell lupin can easily be established in the New Zealand hill and high country, provided that it is scarified, drilled at 1 to 2 cm depth, and more importantly, sown in periods where soil temperatures are within the range of 10 to 20 ^{o}C. Thus, it could be expected that provided soil moisture is adequate, better emergence of Russell lupin will result from sowings in early autumn (March/April) or early to mid spring (September/October).

CHAPTER FOUR

MORPHOMETRICS AND CHARACTERIZATION OF A COLLECTION OF RUSSELL LUPIN GENOTYPES

4.1 INTRODUCTION

As discussed in Chapters 1 and 2, there has been increased interest in searching for alternative pasture species for the New Zealand hill and high country environment. Among possible alternatives, the Russell lupin has proved very successful at low and medium soil fertility levels when grazed by sheep (Scott and Covacevich, 1987). However, there is virtually no published information on the morphology, growth and genetic diversity of Russell lupins.

A few Russell lupin genotypes are held by the Tree and Shrub Improvement Group of the Plant Physiology Division, and in the New Zealand Forage Germplasm Centre of the Grasslands Division, DSIR. There has been no work to characterize these genotypes, and therefore, basic information which could be used in Russell lupin breeding and their agronomic evaluation in New Zealand is limited. To improve the information base on this potential pasture species, the entries in the two germplasm collections were made available for a characterization experiment. The aim of the research was to obtain data to be used in future breeding and agronomic work on this plant in New Zealand. This chapter reports a field experiment which was designed to:-

(i) document and describe the morphology, growth, and performance of Russell lupin;

(ii) characterize the genetic resource, represented by the accessions of Russell lupin from different countries, to obtain basic information;

(iii) to identify and select accessions with potential for increased dry matter production so that these can be examined in more detail.

The study was conducted in Iverson Field on the Lincoln College Research Farm over a one year period from December 1987 to November 1988.

4.2 MATERIALS AND METHODS

4.2.1 Seed source

Seed for the study was acquired from Dr. David Scott of Grasslands Division of DSIR, sub-station Lincoln, Canterbury. Details of the Russell lupin accessions tested and their origins are shown in Table 4.1.

4.2.2 Site description

The soil at the experimental site was a Wakanui silt loam. A soil sample for chemical analysis of the top 15 cm was taken in May 1987. The results of the soil analysis are shown in Table 4.2.

The trial site had been in rape (*Brassica* spp.) during the 1986/87 season after being ploughed out of an established perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture in 1986.

4.2.3 Plot establishment and experimental design

Seed of each accession was sub-sampled and the thousand seed weight determined. Seed was hand scarified by chipping, i.e., the coat of each seed was nicked at the end of the seed opposite to the hilum with nail clippers.

On 6 December 1987, each scarified seed was sown at 1 cm depth in a plastic pot of 200 ml capacity filled with a standard potting mix. After sowing, pots were arranged in wooden boxes (30 pots/box) and placed in a glasshouse. Boxes were inspected and watered daily. Seedling emergence started on the third day (9 December 1987). On 16 December 1987, 15 days after sowing, boxes of seedlings were taken outside to 'harden up' before transplanting into the field.

Table 4.1 Identification and seed sources of the Russell lupin genotypes tested.

Accession number	Accession code	Seed source	1000 seed wt. (g)
Connie	CN	New Zealand	28.9
745	ON	New Zealand	26.0
893	KR	USSR	24.0
894	GR	USSR	23.1
895	QR	USSR	20.6
843	RN	New Zealand	18.5
896	SR	USSR	23.8
897	IR	USSR	23.6
902	HN	New Zealand	24.6
928	FL	Portugal	25.0
932	NG	Germany	23.2
934	DD	Poland	21.3
949	MU	UK	23.3
1043	EG	Germany	25.0
AL2856	PG	Germany	21.3
2923	JD	Poland	22.0
3208	LD	Poland	21.3
3518	BN	New Zealand	26.0

Table 4.2 Soil test results for the Wakanui silt loam used in the characterization study of Russell lupins.

pH	Ca	K	Olsen-P	Mg	S	Na
6.0	9	20	37	30	4	9

The field was ploughed, harrowed and rolled during November and December 1987. No fertiliser was applied, and because of the unknown tolerance of Russell lupin, neither pre- nor post-emergent herbicides were used.

Seedlings were hand transplanted into the field on 22 and 23 December 1987. A randomised Complete block design with three replicates was used (Plates 4.1 and 4.2). Each plot comprised a single row of 10 seedlings of an accession. Each accession of lupin was allocated once, and only once, in each block. However, the naturalised New Zealand Russell lupin, (named Connie in this text), was used as the control and was planted in four plots in each block. This was done to provide a check of site variability within blocks. Additionally two guard rows of Connie were planted at each end of each block. The distance between plots was 1 m, and plants were 0.5 m apart. Plots were therefore 1 m x 5 m with a north to south orientation. A 2 m wide path was left between each block.

All transplanted seedlings were treated with a commercial *Rhizobium* inoculant NZP 2141.

Approximately 24 mm, 32 mm, and 16 mm of water was applied by a sprinkler irrigation in December 1987, January 1988, and February 1988 respectively.

Plots were hand weeded in mid-January, mid-February, mid-March, mid-April, and the last week of August 1988. Further, plots were rotary hoed between lines of plants. Pathways between blocks were mown and clippings removed in late January, mid-March, mid-September, and mid-October 1988.

4.2.4 Data collection

On 10 February 1988, five well established plants from each plot were identified and marked. Throughout the study period, data collection was confined to these sample plants. Measurements were done on individual plants. Characters were chosen to include and summarize the size, shape and growth of each plant. Flowering characteristics and preliminary observations on animal acceptability were also included. Data were collected

Plate 4.1 General view of the Lincoln College trial with different Russell lupin genotypes on 10 February 1988 (48 days after transplanting).

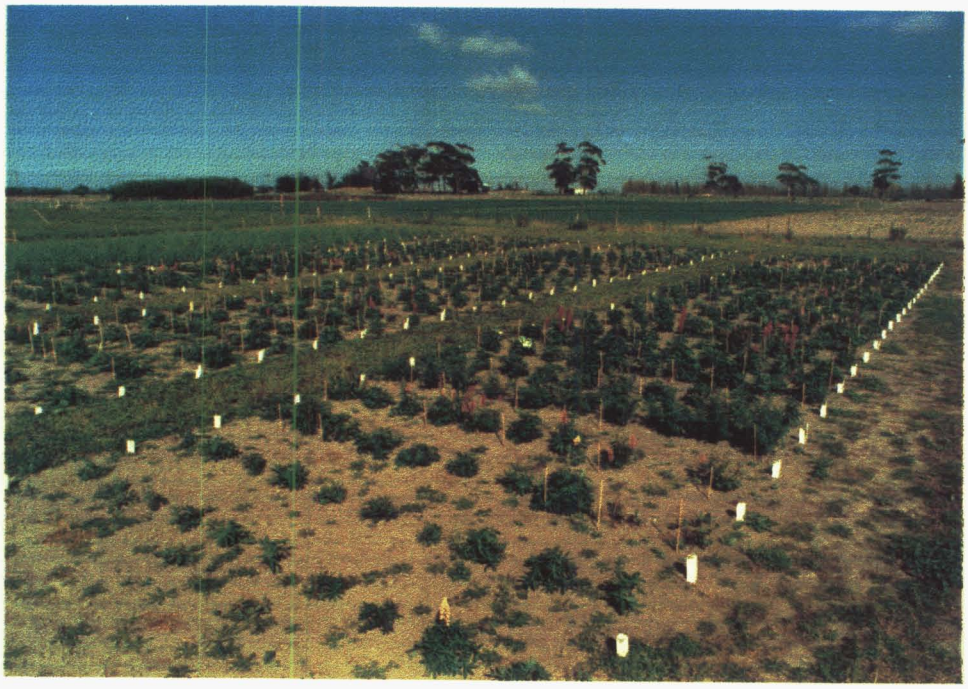


Plate 4.2 General view of the Lincoln College trial with different Russell lupin genotypes on 21 March 1988 (88 days after transplanting).

on two occasions, once each in autumn and in spring 1988, although regular observations were made throughout the experiment.

Morphological characteristics were measured *in situ* and were collected between 8-11 March 1988 (for the autumn season), and between 15-17 November 1988 (for the spring season). When the autumn measurements were made, most plants were vegetative. Thus, leaf dimension and leaflet number were measured on a randomly selected leaf of each sample plant, and were thus rosette leaves. However, at the time of the spring measurements, almost all genotypes had flowered. Leaf dimension and leaflet number were therefore taken on the leaf located at two nodes below the main inflorescence, and were thus stem leaves.

Plant characteristics measured were:-

(i) Leaflet number:- of a randomly selected leaf (autumn), or a leaf located two nodes below the main inflorescence (spring).

(ii) Leaf diameter:- of the leaf in (i), to the nearest 0.1 cm.

(iii) Leaflet length:- of the middle largest leaflet of the leaf in (i), to the nearest 0.1 cm.

(iv) Leaflet width:- of the leaflet in (iii), to the nearest 0.1 cm.

(v) Plant height :- height from the ground level was measured to the nearest 1 cm, on both occasions.

(vi) Plant spread (plant width):- the horizontal spread of the plant covering the ground was measured to the nearest 1 cm, on both occasions.

(vii) Raceme length:- was measured during mid-flowering and was recorded to the nearest 1 cm, as the distance between the lower most flower to the upper most flower on the main inflorescence.

(viii) Stem width:- was measured at the base of the main inflorescence, approximately 3 cm above ground level, and was recorded to the nearest 0.1 cm.

(ix) Production of flowers before exposure to cold and/or short days was scored as:-

0 - no, &

1 - yes.

(x) Inflorescence status:- was scored during mid-flowering and the following scoring procedures were followed based on the number of racemes per plant:-

1 - when there was single raceme;

2 - when there were 2-3 racemes ; and

3 - when there were >3 racemes.

(xi) Flower colour:- the colour of the flower was recorded during mid-flowering of each plant.

(xii) Flowering date:- was recorded when at least one open flower appeared on each plant. Days to first flowering were calculated from 21 December (the longest day in New Zealand).

(xiii) Plant habit:- was observed during mid-flowering and was recorded as erect, semi-erect, ascendant, or decumbent.

(xiv) Dry matter yield:- was measured at two harvests carried out between 27 and 29 May 1988 in autumn, and between 22 and 24 November 1988 in spring. At both harvests, sample plants were cut to ground level using hand clippers and all above ground plant material was oven dried. Dry matter yield per plant was recorded to the nearest 0.1 g.

(xv) Preliminary observation on acceptability to stock:- was observed on the plants that were left in the field after sample plants had been harvested for yield determination. On both occasions, sheep were conditioned to grazing lupins on a separate 0.8 ha. block of Connie lupin. After three days on the Connie block, animals were transferred to the experimental site to graze the different Russell lupin accessions. On 17 June 1988 and 5 December 1988, the plots were scored for their acceptability to sheep from 5 (untouched) to 1 (preferred).

(xvi) Disease and pest incidence:- regular observation was carried out throughout the study period.

An initial germination and emergence test should have been the first step of this study. However, the previous conditions governing the seed, especially storage and age, were likely to have been different for the different accessions. Thus, germination and

emergence tests may not have shown the true genetic differences among the accessions and were excluded from the experiment.

Because of the different seed ripening period-both within and among accessions-and problems of pod shattering, measurements related to pod and seed production were not included.

Variation in the leaf alkaloid level of the different accessions was studied by Gibbs (1988), as part of an Honours dissertation. For this study, leaf material was taken from sample plants on 14 March, 12 April, 10 May 1988 (in autumn), and on 29 September 1988 (in spring). On all occasions, about 8 g fresh weight (1-2 leaves) of leaf material was randomly cut from each sample plant.

4.2.5 Data analysis

All plant characteristics included in this study, except plant habit, were subjected to separate frequency distribution. These were used to show the range of morphological and agronomical variation in the collection of Russell lupin plants as a sample of the population. Besides these, separate analyses of variance were calculated for all variables except inflorescence status, flower colour, plant habit and autumn flowering. These analyses were done to improve the information base of the morphological and growth performance of Russell lupin accessions among single variates.

The data was further analysed by multivariate analysis of variance. Once the multivariate statistical test of any and every difference among the class (accession) means was shown to be highly significant ($p \leq 0.001$) among the accessions, canonical variate analysis was performed. All measurements, except inflorescence status, flower colour, leaf dimensions in spring, leaflet number in spring and plant habit, were included in the latter analysis. Canonical variate analysis was performed using the CANDISC Procedure of the Statistical Analysis System Programme (SAS Institute, 1985). Review of the canonical variate analysis is presented in Sections 4.2.6 and 4.2.7. However, the following steps were generally considered during the data analysis and interpretation stage.

- Partial correlation coefficients of the plant characteristics included in this analysis were calculated and were used to show the relationship between variables.
- New canonical variates were computed from the original variates to discriminate most effectively among the variates.
- An F-approximation was used to test the hypothesis that each and every canonical correlation was zero in the population. The significance level for this test was taken to be $P \leq 0.05$, and was used to draw conclusions on the required number of canonical variates that were worthy of consideration.
- The between-accession variability represented by each canonical variate was reported as a percent of the proportion of eigen values of each canonical correlation over the sum of eigen values of all canonical correlations.
- Because the plant characteristics included in this study could not be measured in the same units, the standardised canonical coefficients, rather than the raw canonical coefficients, were interpreted. Those coefficients with a value about 50% or more of the greatest canonical coefficient were considered as aids to interpretation of a canonical variate.
- The relationship among the 18 accessions was displayed by a two or three dimensional scatterplot of the canonical variates, in which co-ordinate axes corresponding to the canonical variate means of each accession of Russell lupin.
- The 95% confidence region of each accession was calculated by circles with a radius of $1.96/\sqrt{n}$, where n was the number of sample plants in each accession (Seal, 1966; Chatfield and Collins, 1980).

4.2.6 Canonical variate analysis

Canonical variate analysis is a multivariate statistical technique, which is used for data reduction or summarizing and interpretation of data (Seal, 1966; Pearce, 1969; Blackith and Reyment, 1971; Srivastava and Carter, 1983). Canonical variate analysis is an extension of the method of discriminant functions and generalised distances in multidimensional space (Seal, 1964; Pearce, 1969; Blackith and Reyment, 1971). This statistical technique is also related to the other multivariate statistical techniques: principal

component analysis (PCA) (Pearce, 1969; SAS Institute, 1985) and canonical correlation analysis (SAS Institute, 1985).

Given a classification variable (two or more groups of observations) and several quantitative variables, canonical variate analysis computes a linear combination of the quantitative variables, called canonical variates (components) (Mardia *et al.*, 1979; Chatfield and Collins, 1980; SAS Institute, 1985). A canonical variate has the highest possible multiple correlation with the classification variable or groups, and summarizes the between-accession variation. In the computational procedure, the maximal multiple correlation is called the first canonical correlation, and the variable defined by the linear combination is the first canonical variable or canonical component. Each canonical variable has coefficients equal to the canonical coefficients or canonical weights.

The second canonical correlation is obtained by finding the linear combination uncorrelated with the first canonical variable that has the highest possible multiple correlation with the groups. The process of computing canonical variables can be repeated until the number of original variables or the number of classes minus one, which ever is smaller. Whether the correlation is calculated from the total sample or from the pooled within-class correlation, canonical variates remain uncorrelated.

4.2.7 The identification of important canonical variates and interpretation of canonical coefficients

Although **X** canonical variates are required to explain the total between-class variability, often much of the between-class variability is accounted for by a few of the canonical variates (**P**). After calculating the canonical correlation, the usual procedure is to look at the first few canonical correlations, which hopefully account for the highest possible multiple correlation with the classification variable. The usual way of looking at the relative importance of canonical variates relies on carrying out an F approximation (test) of the null hypothesis that all the canonical correlations are zero in the population (SAS Institute, 1985). The advantage of this procedure is to assess which of the canonical variates are significant and worthy of consideration.

The first canonical correlation is at least as large as the multiple correlation between the groups and any of the original variables. If the original variables have low within-group correlations, then the first canonical correlation is not much greater than the largest multiple correlation. If the original variables have high within-group correlations, the first canonical correlation can be large even if all the multiple correlations are small. In other words, the first canonical variable can clearly show substantial differences among the classes even if none of the original variables do.

Once the required number of canonical correlation(s) is identified, then the next procedure is to identify the most important quantitative variables that can help explain the canonical variates. This is possible by examining the canonical coefficients or canonical weights. The interpretation of canonical coefficients for a given canonical variate relies on considering those quantitative variables which have relatively high positive and/or negative weighting as constituting as an index of the combined action, or contrast of the original variables.

However, the canonical coefficients are not orthogonal, so the canonical variables do not represent the perpendicular directions through the space of the original variables. It is customary to normalise (standardise) the canonical coefficients so that the pooled within-group variance of the canonical variable is one. This is recommended where the measurements are not made in the same units (SAS Institute, 1985).

4.3 RESULTS

4.3.1 Climate data

The monthly rainfall recorded approximately 4 km from the experimental site, from December 1987 to November 1988, is presented in Figure 4.1.

Relative to the mean annual rainfall for the area (650 mm), very little rain fell and moderately dry to very dry conditions prevailed throughout almost all the experiment. Windy, sunny, and warm days gave above average evaporation, transpiration and moderately dry to very dry soils.

The annual rainfall during the study period totalled 319 mm and this was the driest year since records began in 1880.

The monthly average photoperiod and the maximum and minimum air temperature recorded are presented in Figure 4.2. The change in photoperiod was similar in shape to the monthly average change in air temperature. Over all, the months from April to August had shorter photoperiods (Figure 4.2a).

Maximum temperatures were generally above the long term average, although the months of April and May were near average maximum temperatures (Figure 4.2b). Compared to the average maximum temperature for the area, the experiment was conducted during a mild winter, with a hot spring and hot summer (Figure 4.2b).

Minimum temperatures were similar to the long term average, except in the autumn and early winter months which experienced colder nights (Figure 4.2b).

4.3.2 Morphology and growth of Russell lupin

Russell lupins have epigeal germination, where the cotyledons are carried above the soil surface (Plate 4.3). The cotyledons eventually wither and fall off.

Growth habit

The plants exhibited a herbaceous growth habit with a semi- erect plant habit. By maturity, plants in the collection had up to 120 cm height. The frequency distribution for plant height was skewed to the right in early autumn, with few plants growing taller than 65 cm (Figure 4.3a). In spring however, the frequency distribution for plant height was skewed to the left, with few plants shorter than 65 cm (Figure 4.3b). Although, most plants (85%) reached a height of 75-115 cm at maturity (Figure 4.3b), accessions ON, EG, NG, PG, JD, LD and GR were highly significantly ($p<0.001$) taller than BN, RN, IR, FL, KR and SR (Table 4.3).

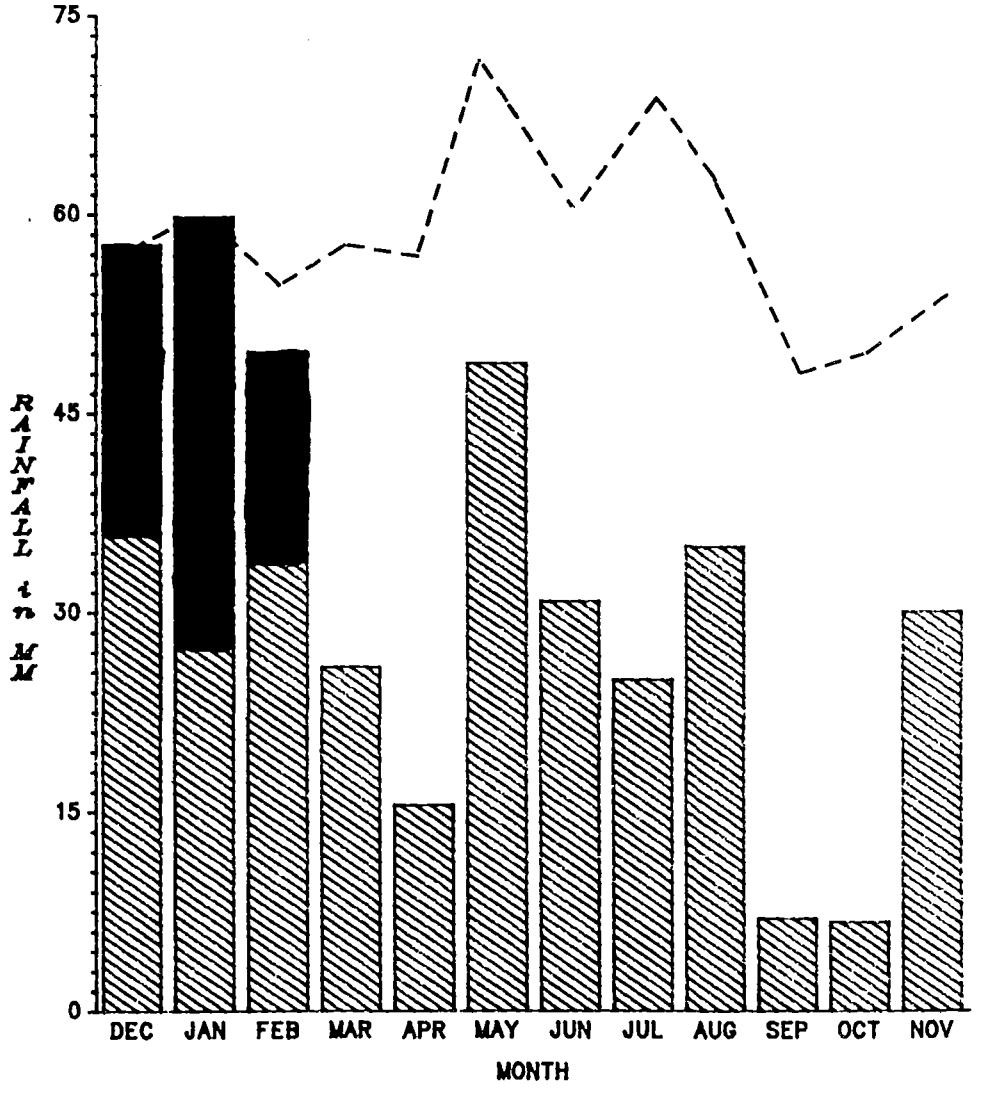


Figure 4.1 Monthly rainfall (striped bars) and irrigation applied (closed bars) during experiment and average rainfall over 1930 to 1981 (broken line).

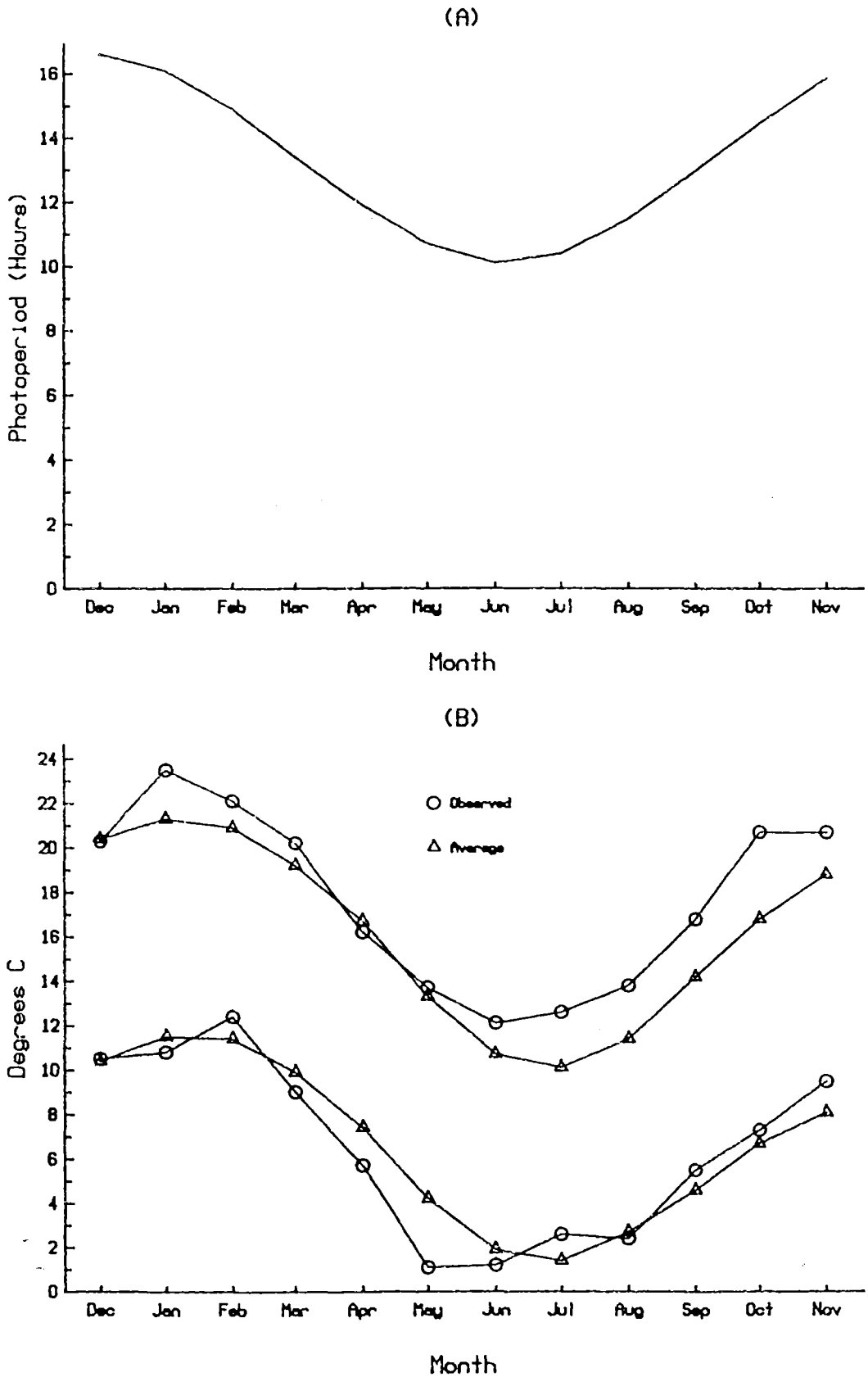


Figure 4.2. Monthly (A) photoperiod and (B) maximum and minimum temperature during the experiment and average temperature (1967 to 1977)

Plate 4.3 Epigeal germination of Russell lupins, i.e., cotyledons are carried above the soil surface.

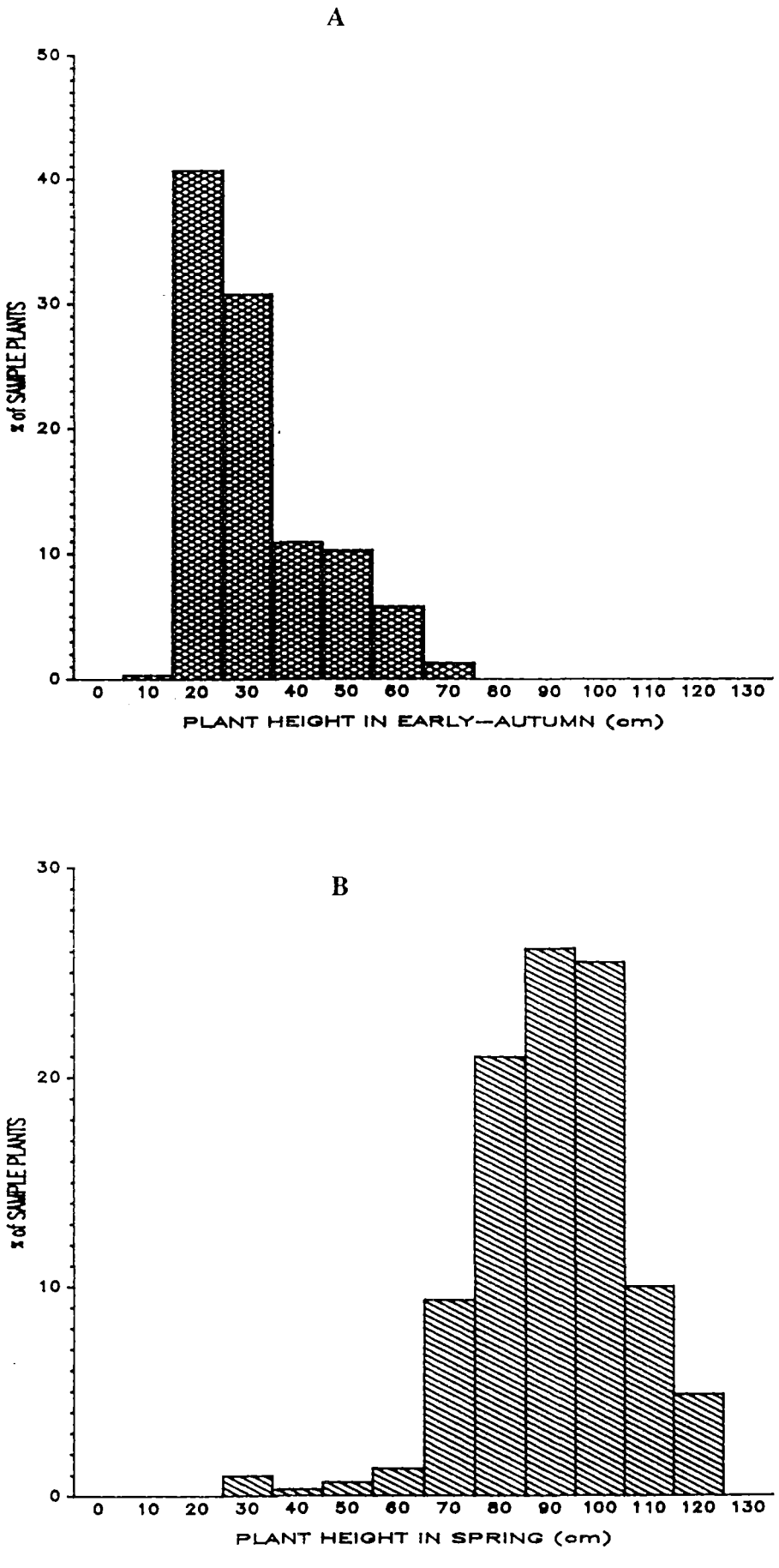


Figure 4.3. Frequency distributions of (A) early autumn and (B) spring plant height in 312 sample Russell lupin plants in the germplasm collection.

Table 4.3. Plant height, plant spread, stem width, leaflet number, and leaf dimensions of Russell lupin accessions grown in Canterbury from December 1987 to November 1988.

Accession	Plant height (cm)		Plant spread (cm)		Stem width (cm)	Leaflet number		Leaf diameter (cm)		Leaflet Length (cm)		Leaflet Width (cm)	
	AUT	SPR	AUT	SPR		AUT	SPR	AUT	SPR	AUT	SPR	AUT	SPR
BN	35	71	40	73	1.2	10	13	16.9	12.4	8.6	6.6	2.2	1.6
CN	36	87	44	84	1.2	11	13	17.4	12.7	9.1	6.9	2.4	1.7
DD	34	88	45	80	1.2	12	13	19.8	13.1	10.8	7.4	2.4	1.5
EG	27	104	45	89	1.2	12	12	21.0	14.3	11.1	7.8	2.5	1.8
FL	28	85	42	84	1.4	12	13	18.7	11.8	9.6	6.5	2.7	1.7
GR	27	95	46	84	1.0	12	13	22.1	12.8	11.4	7.1	2.7	1.5
HN	48	93	43	78	1.3	11	13	18.5	13.5	9.8	7.2	2.6	1.7
IR	27	85	42	75	1.0	12	13	20.4	13.1	10.9	7.2	2.4	1.5
JD	25	95	42	86	1.1	13	13	20.0	14.0	10.6	7.6	2.3	1.6
KR	29	85	44	79	1.2	12	13	22.1	12.6	11.7	7.2	2.8	1.6
LD	27	95	41	82	1.2	12	13	20.9	13.1	11.2	7.5	2.5	1.6
MU	25	93	45	90	1.4	12	12	20.9	13.2	10.9	7.5	2.6	1.8
NG	31	97	47	91	1.3	12	12	21.5	13.8	11.1	7.7	3.0	1.8
ON	28	106	50	91	1.3	13	13	20.3	13.0	10.6	7.1	2.7	1.7
PG	34	97	43	79	1.1	11	13	20.1	12.8	10.4	7.3	2.5	1.4
QR	21	89	40	81	1.1	12	13	19.3	14.0	10.1	7.5	2.3	1.7
RN	27	81	38	74	1.1	11	12	17.1	12.7	8.8	7.0	2.2	1.7
SR	28	82	44	73	1.0	12	12	19.8	11.6	10.7	6.8	2.5	1.4
Significance	***	***	***	***	***	***	ns	***	*	***	ns	***	ns
CV (%)	34.4	13.8	13.5	15.3	21.6	12.9		16.3		16.0		20.5	

ns - not significant at 5% probability level;
*, **, and *** - significantly different at the 5, 1, and 0.1% probability levels respectively;
AUT - measurements taken in early-autumn (early March);
SPR - measurements taken in late-spring (mid November).
Accession refers to accession codes given in Table 4.1.

Horizontal spread

The horizontal spread of the plants in early autumn varied from 15 to 65 cm (Figure 4.4a). In spring the range of plant spread was nearly twice as much (Figure 4.4b). By maturity, 90% of the plants had a horizontal spread of 65 to 115 cm, although the modal class was 80 cm (Figure 4.4b). In the spring, accessions ON, NG and MU had highly significantly ($p<0.001$) greater horizontal plant spread than BN, SR, RN and IR (Table 4.3).

Stem

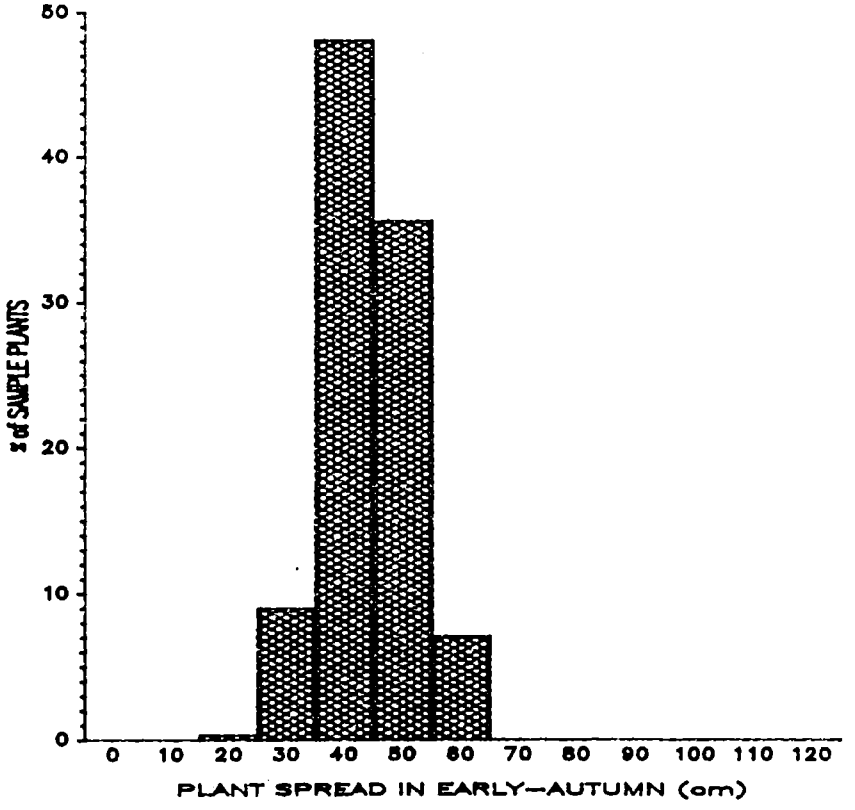
In Russell lupin, the stem is semi-erect, mostly unbranched, fistulose (hollow) and inconspicuous until flowering. Stem thickness ranged between 0.5 to 2.1 cm, although most plants (85%) had a stem diameter of 0.9-1.5 cm (Figure 4.5). The stem width of FL, MU, ON, NG and HN was highly significantly ($p<0.001$) wider than GR, IR and SR (Table 4.3).

Leaves

The plants had palmate leaves with 5-16 leaflets per leaf. The elliptical shaped leaflets were glabrous above and sparsely hairy below. The frequency distributions of leaflet number showed that most plants, (85%), had 10-14 leaflets, although the modal class was 12 cm (Figure 4.6a,b). Accession codes ON and JD had the highest number of leaflets in early autumn (Table 4.3). However, leaflet number in spring was not significantly different among accessions (Table 4.3).

The leaf dimension of Russell lupins changed with growth stage and leaves were generally longer and wider in the rosette stage than leaves formed on the main inflorescence (stem leaves). Overall, leaf diameter ranged from 7 to 29 cm, and the modal classes of leaf diameter were 20 and 12 cm in rosette and stem leaves respectively (Figure 4.7a,b). In early autumn NG, KR, MU, LD and PG had highly significantly ($p<0.001$) wider rosette leaf diameter than BN, RN and CN; but in spring, EG, JD and QR had significantly ($p<0.05$) wider stem leaves than the other genotypes (Table 4.3).

A



B

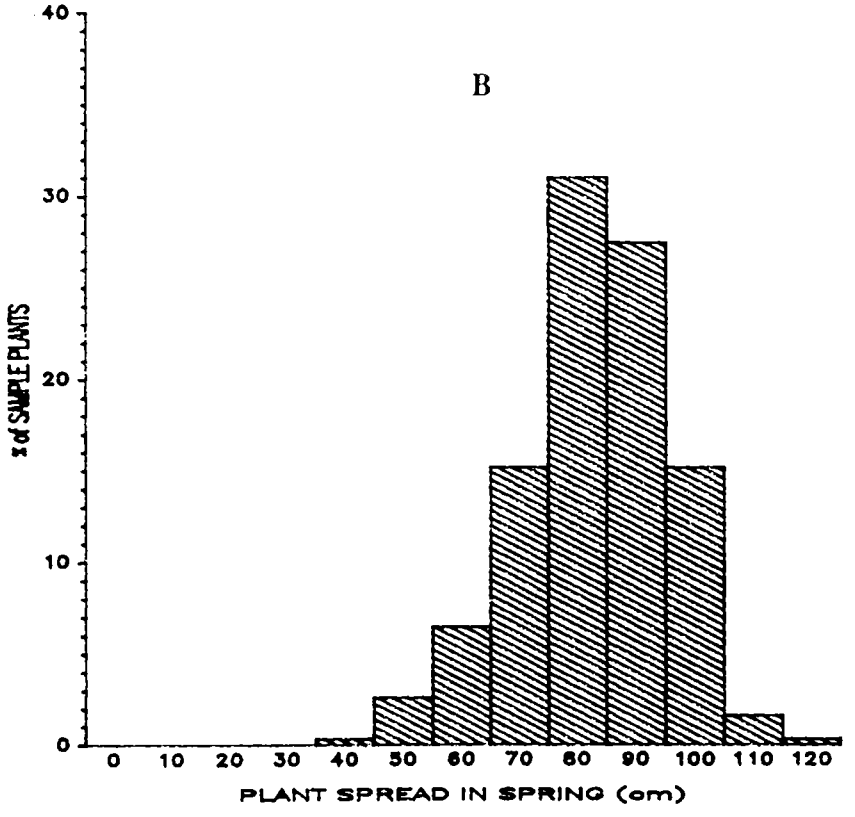


Figure 4.4. Frequency distributions of (A) early autumn and (B) spring plant spread in 312 sample Russell lupin plants in the germplasm collection.

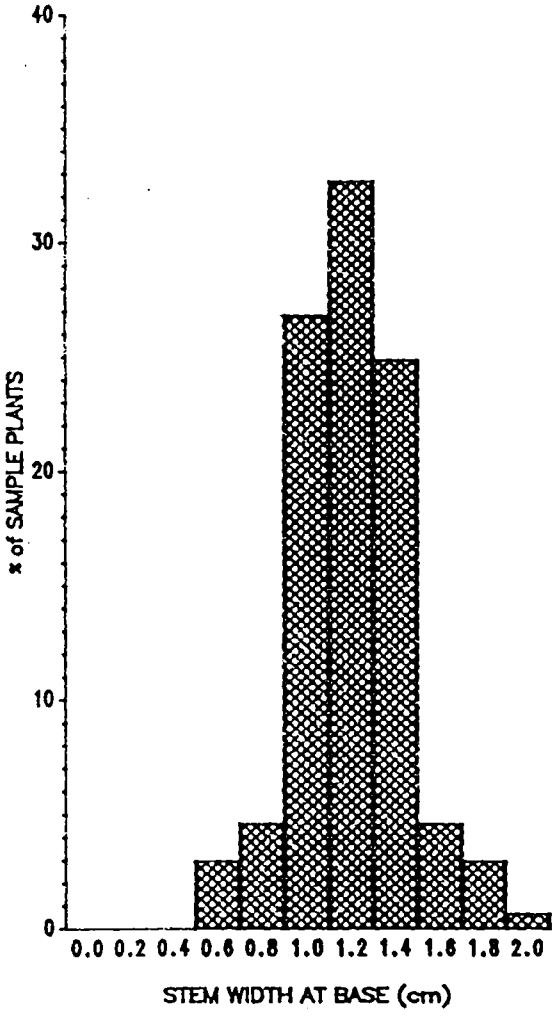


Figure 4.5. Frequency distribution of stem width at base in 312 sample Russell lupin plants in the germplasm collection.

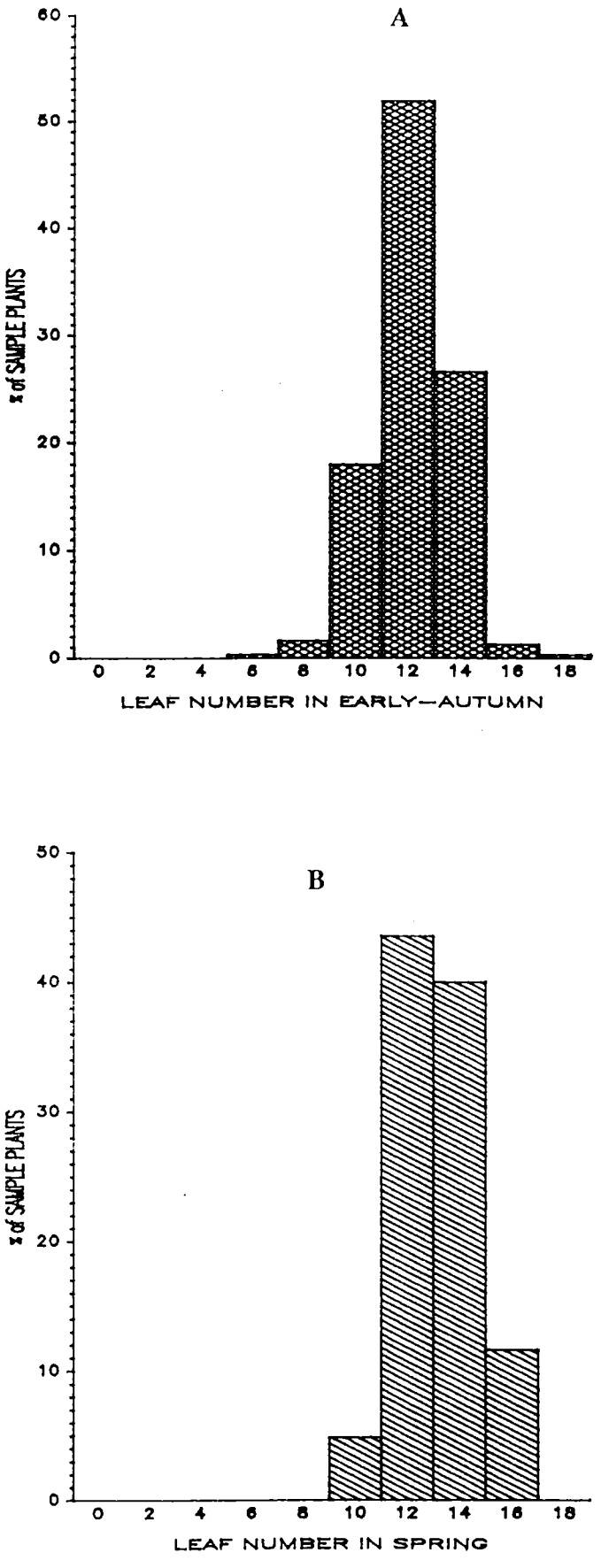
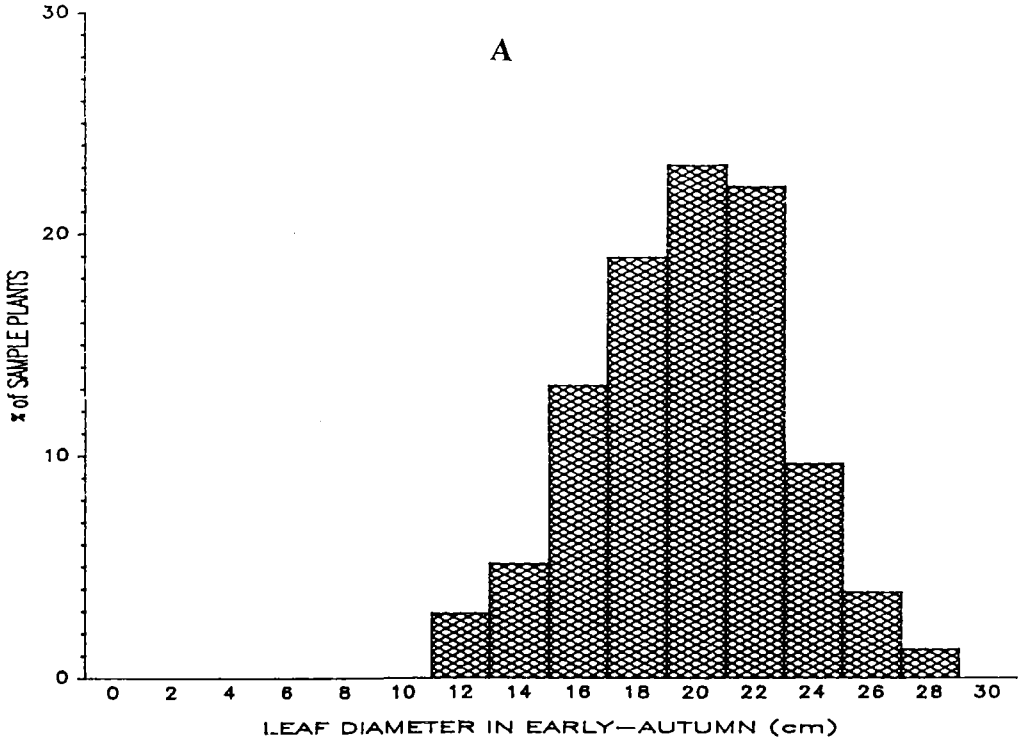


Figure 4.6. Frequency distributions of leaflet number in (A) early autumn and (B) spring in 312 sample Russell lupin plants in the germplasm collection.



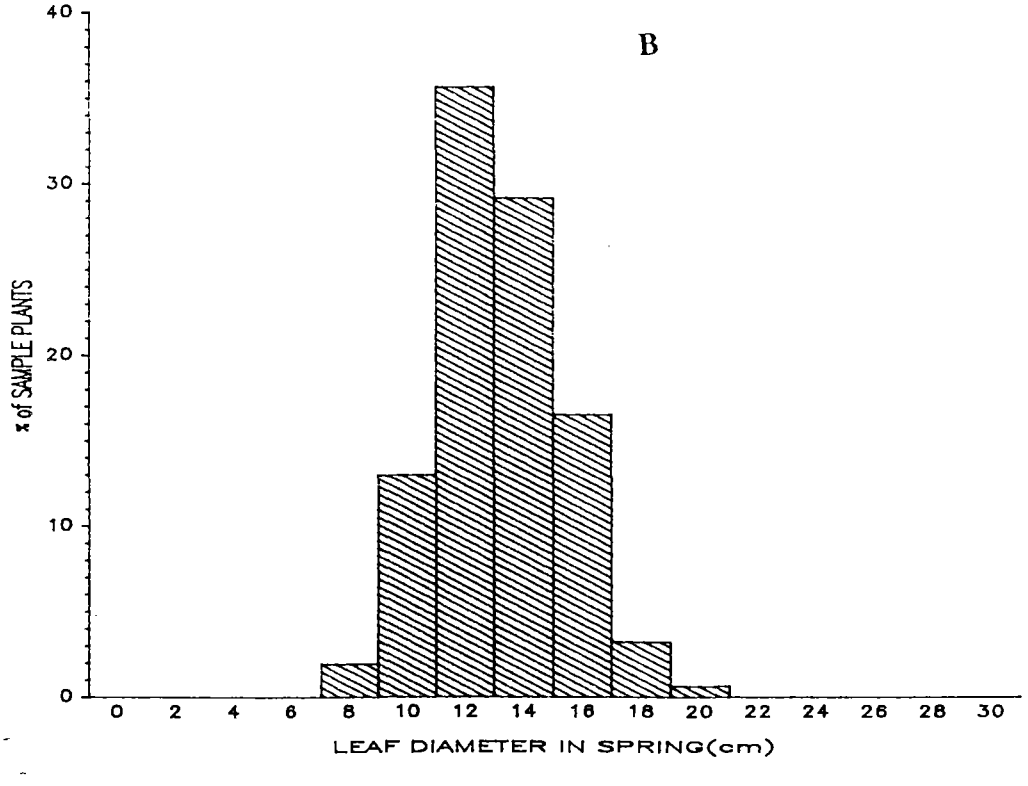


Figure 4.7 Frequency distributions of (A) early autumn and (B) spring leaf diameter in 312 sample Russell lupin plants in the germplasm collection.

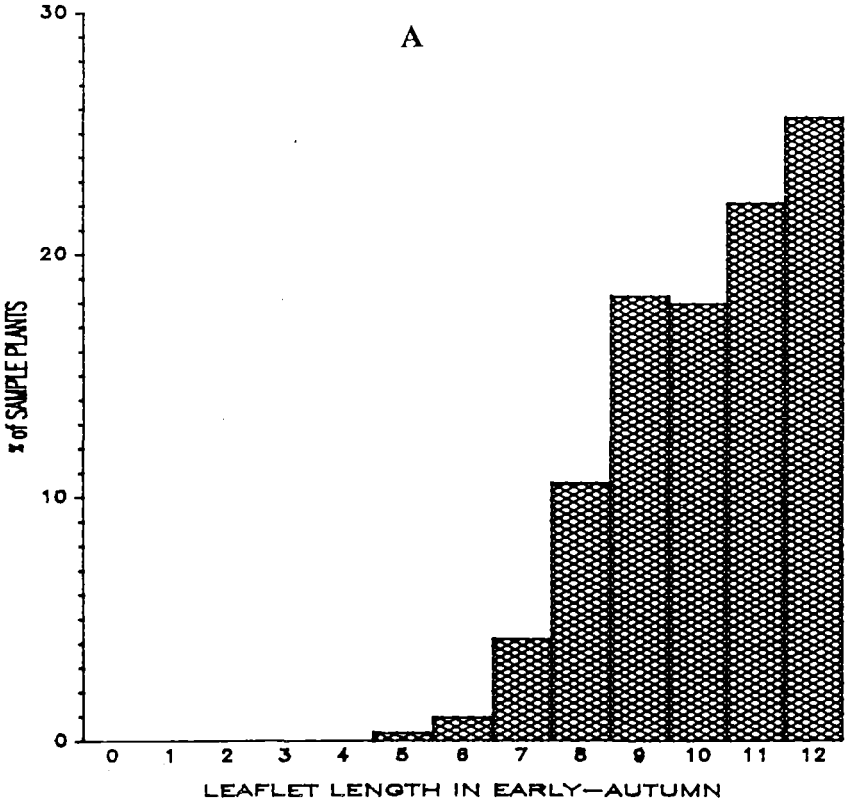
In rosette leaves, the largest leaflets were 5-13 cm long (Figure 4.8a), and 1.5-4.5 cm wide (Figure 4.9a); whilst leaflets formed on the main inflorescence were 4-11 cm long (Figure 4.8b), and 1-3 cm wide (Figure 4.9b). The frequency distributions of leaflet length showed modal classes of 11 and 7 cm in rosette and stem leaves respectively. The modal classes for leaflet width, consisting of about half of the plants, were 2.5 and 1.5 cm respectively in the rosette and stem leaves. In early autumn genotypes KR, GR, NG, ON and MU had highly significantly ($p<0.001$) longer and wider rosette leaflets than BN and RN (Table 4.3). There was no significant difference among the accessions in stem leaflet length and width (Table 4.3).

Flowers

Russell lupin flowers are borne on an elongated terminal raceme of up to 60 cm long. While the raceme length of plants in the collection varied from 15-60 cm, 75% of sample plants had racemes of 30-40 cm (Figure 4.10a). The modal class was 35 cm. Raceme length was highly significantly ($p<0.001$) different among the accessions and MU, PG, QR, EG, GR and JD had longer racemes than the other accessions (Table 4.4). Plants were observed to form multiple racemes (>3 per plant), although one out of twenty plants had single or 2-3 racemes per plant (Figure 4.10b).

The flower colour of plants in the collection was highly variable and various degrees of blue, pink, purple, maroon, yellow, white, and combinations of pink and white, pink and yellow, purple and white and purple and yellow were exhibited (Plate 4.4 - 4.6). However, about 60 and 20% of the plants in the collection had blue and pink flowers respectively (Figure 4.11a).

Days to first flowering was also variable. About 40% of plants in the collection flowered within three months of transplanting (Figure 4.11b). However, the tendency to produce flowers and pods on some plants was stopped soon after mid-March. On these plants, new leaves emerged in place of the buds and flowers on the raceme (Plate 4.7). Between 270 to 300 days after transplanting were required for 55% of the plants in the collection to commence flowering (Figure 4.11b). Nevertheless, almost all plants in the



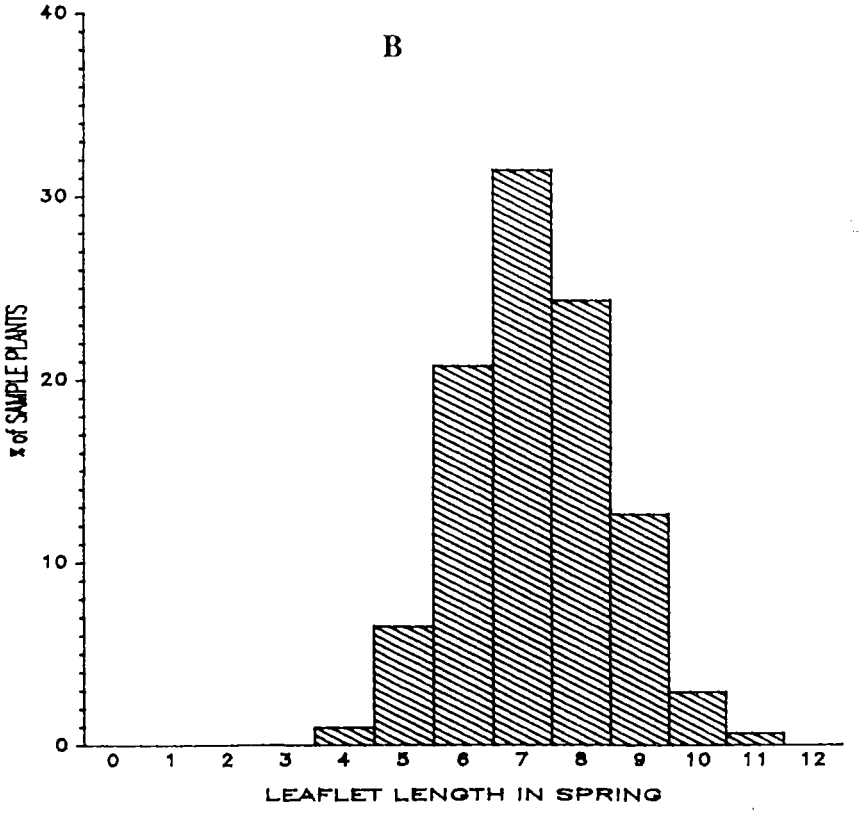


Figure 4.8. Frequency distributions of (A) early autumn and (B) spring leaflet length in 312 sample Russell lupin plants in the germplasm collection.

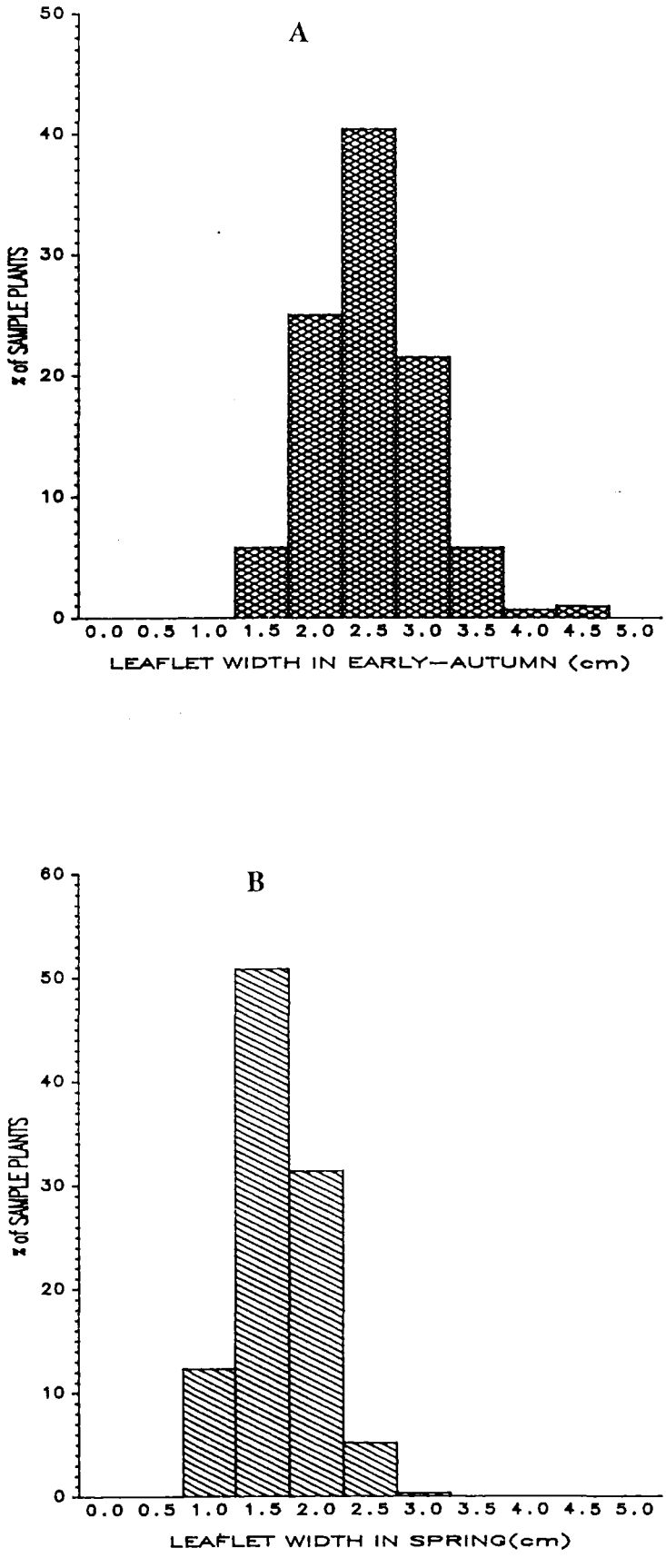
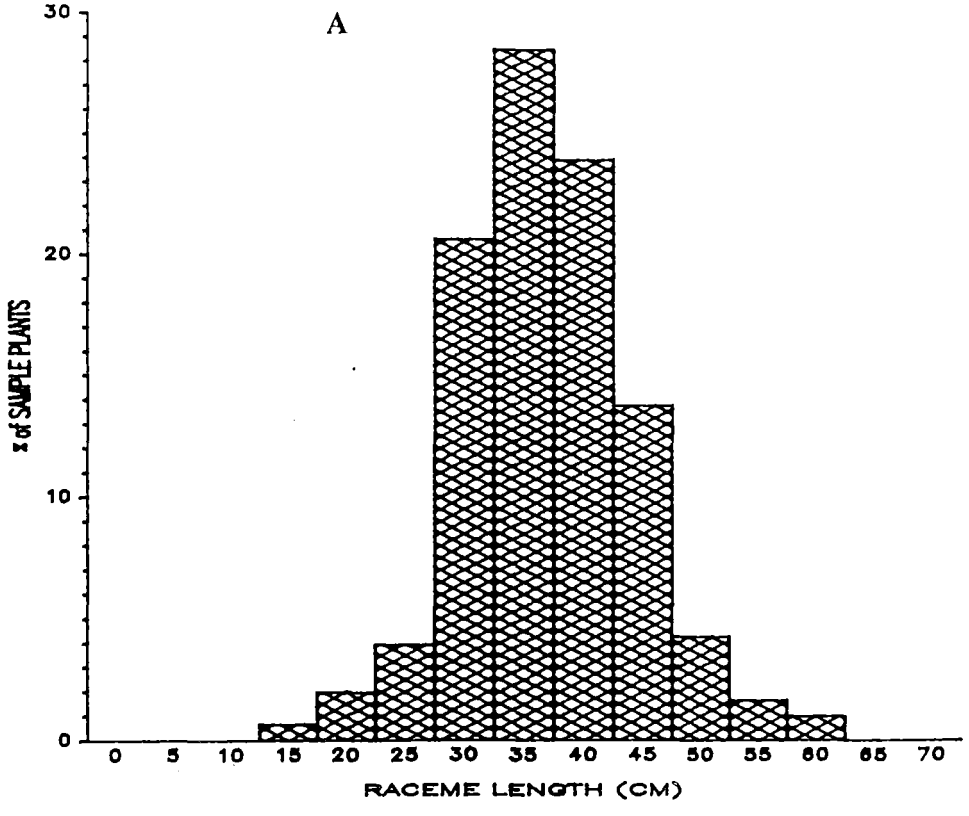


Figure 4.9. Frequency distributions of (A) early autumn and (B) spring leaflet width in 312 sample Russell lupin plants in the germplasm collection.



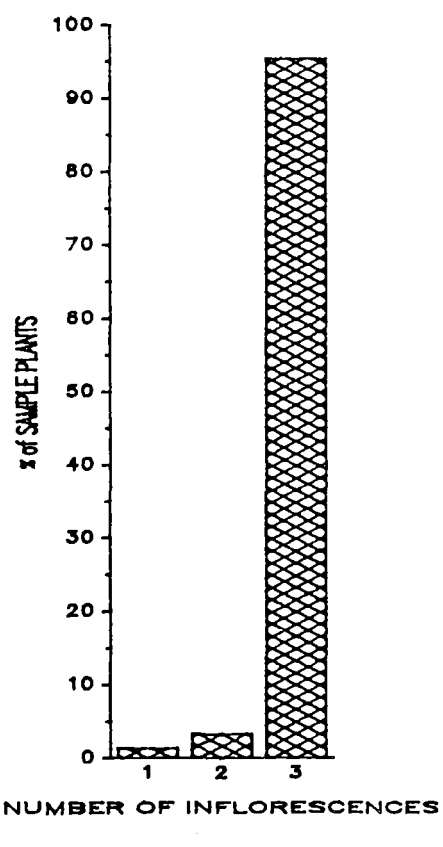


Figure 4.10. Frequency distributions of (A) raceme length, and (B) inflorescence status, in 312 sample Russell lupin plants in the germplasm collection.

Table 4.4. Raceme length, days to first flowering and drymatter yield of Russell lupin accessions grown in Canterbury from December 1987 to November 1988.

accession	Raceme length(cm)	Days to first flowering	Dry matter yield plant^{-1} (g) AUT	SPR	TOTAL
BN	30	129	33.5	185.4	218.9
CN	35	138	49.6	286.9	336.5
DD	37	211	33.1	251.2	284.3
EG	40	271	37.0	315.6	352.6
FL	33	246	46.2	266.0	312.2
GR	40	273	31.7	292.0	323.7
HN	37	112	70.3	296.9	367.2
IR	35	252	16.9	179.0	195.9
JD	39	261	32.2	292.8	325.0
KR	36	217	40.1	221.2	261.3
LD	38	280	29.6	267.9	297.5
MU	40	242	45.2	301.4	346.6
NG	39	184	48.6	336.4	385.0
ON	38	253	43.3	363.1	406.4
PG	40	205	36.6	256.6	293.2
QR	39	281	31.7	295.0	326.7
RN	37	190	44.1	272.0	316.1
SR	37	220	29.1	212.4	241.5
Significance	***	***	***	***	***
CV (%)	19.3	46.8	52.8	34.1	13.2

*** - significantly different at 0.1% probability level;

AUT - measurements taken in early autumn (early March);

SPR - measurements taken in late spring (mid November).

Accession refers to accession codes given in Tale 4.1.

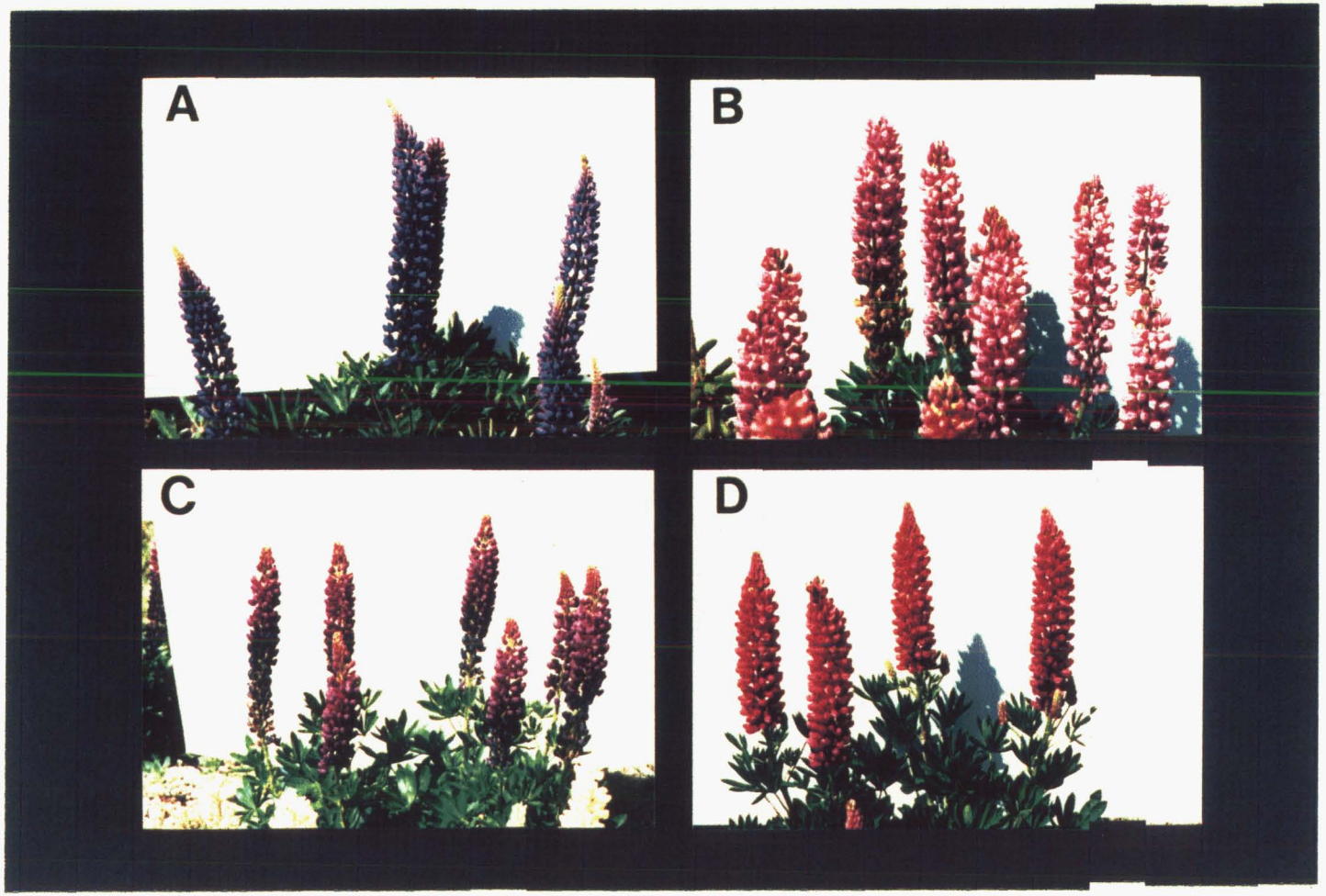


Plate 4.4 The flower colour was variable and exhibited various degrees of (A) Blue, (B) Pink, (C) Purple, (D) Maroon

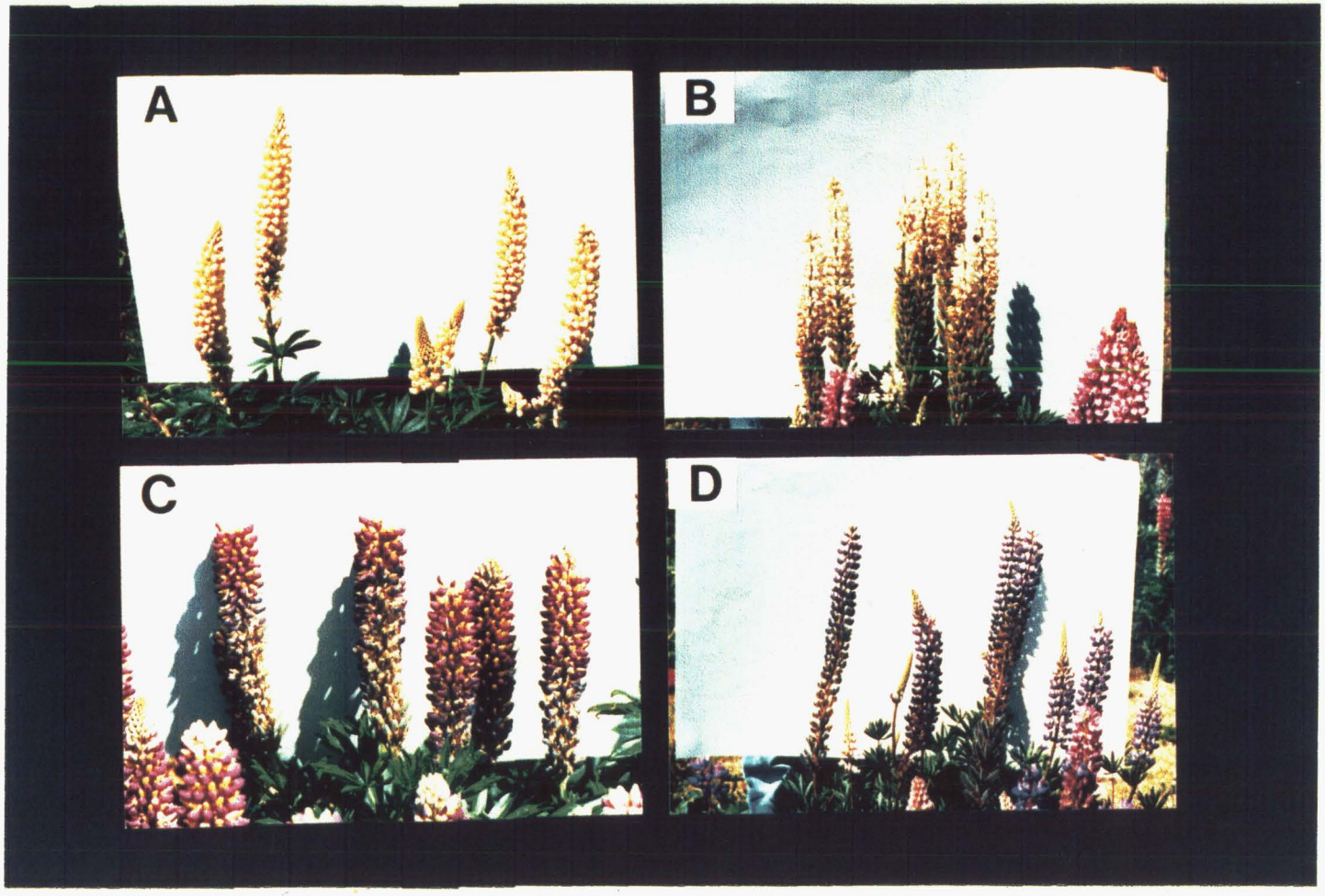


Plate 4.5 Flower colour continued.... (A) Yellow, (B) White, (C) Purple and yellow, (D) Purple and white.

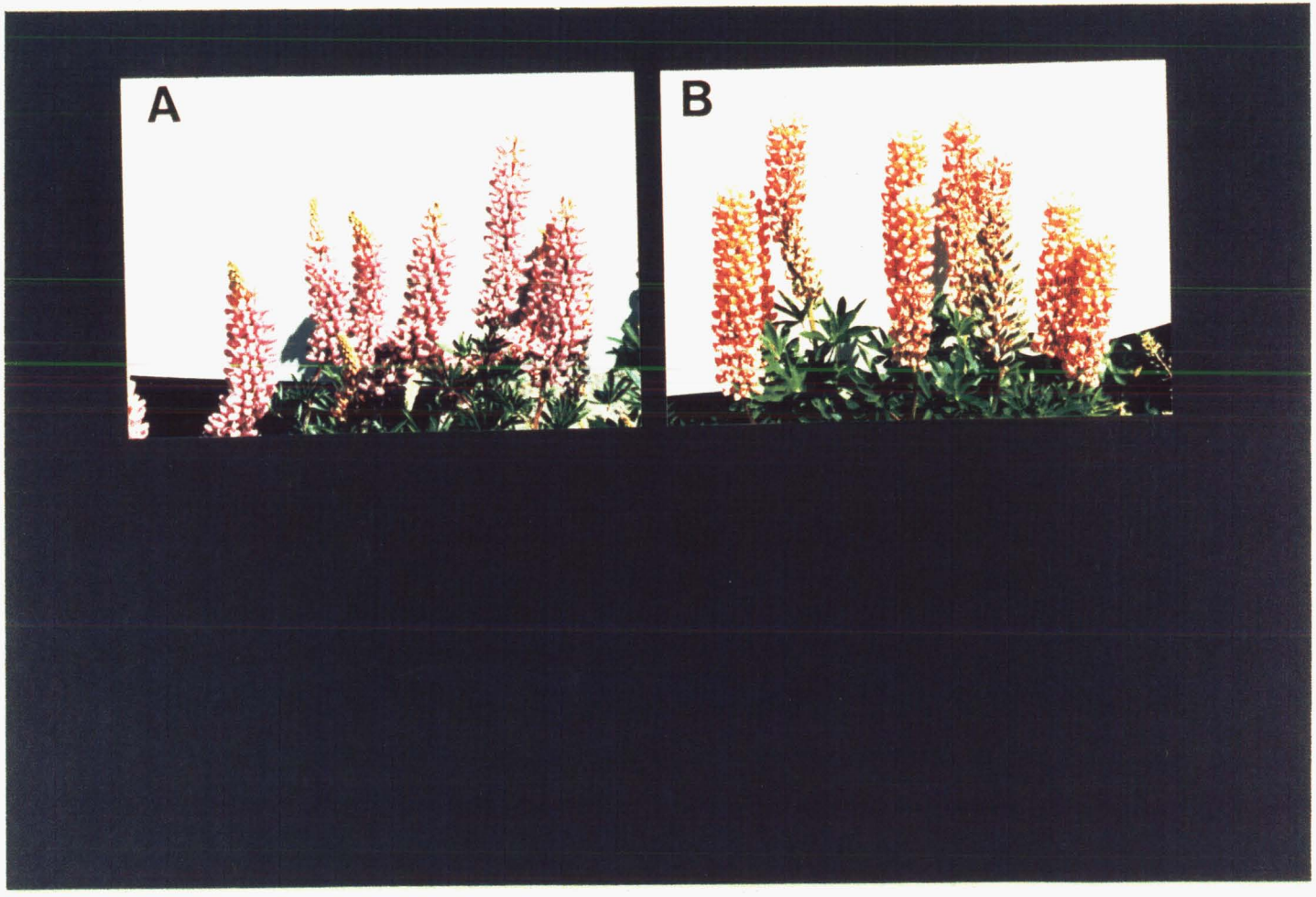
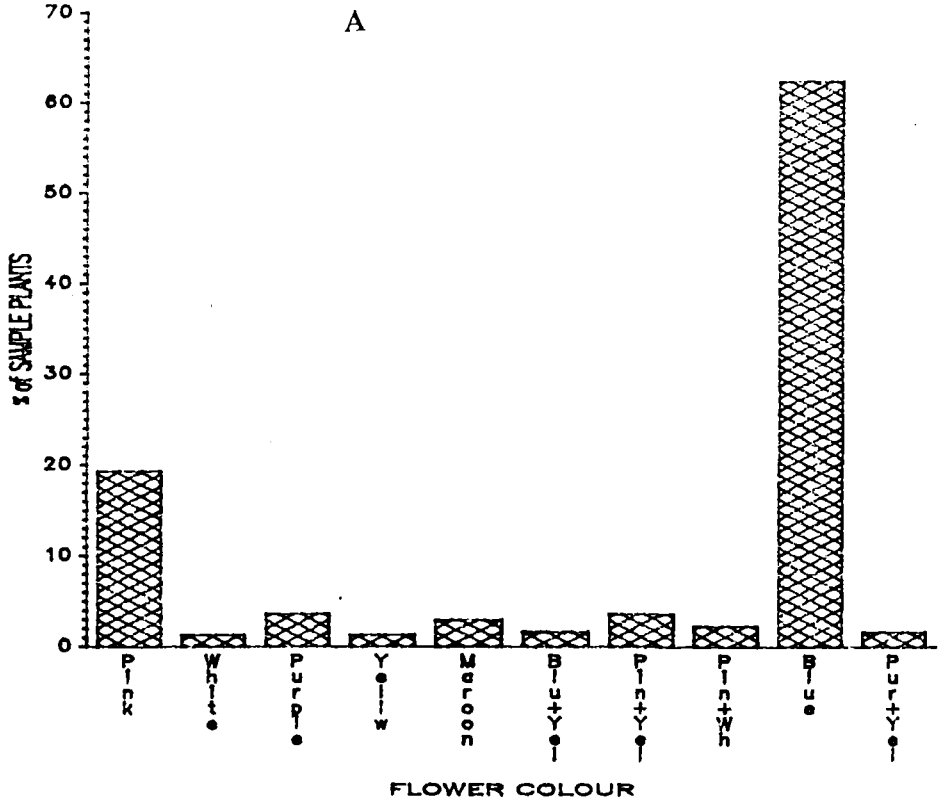


Plate 4.6 Flower colour continued.... (A) Pink and white, and (B) Purple and white.



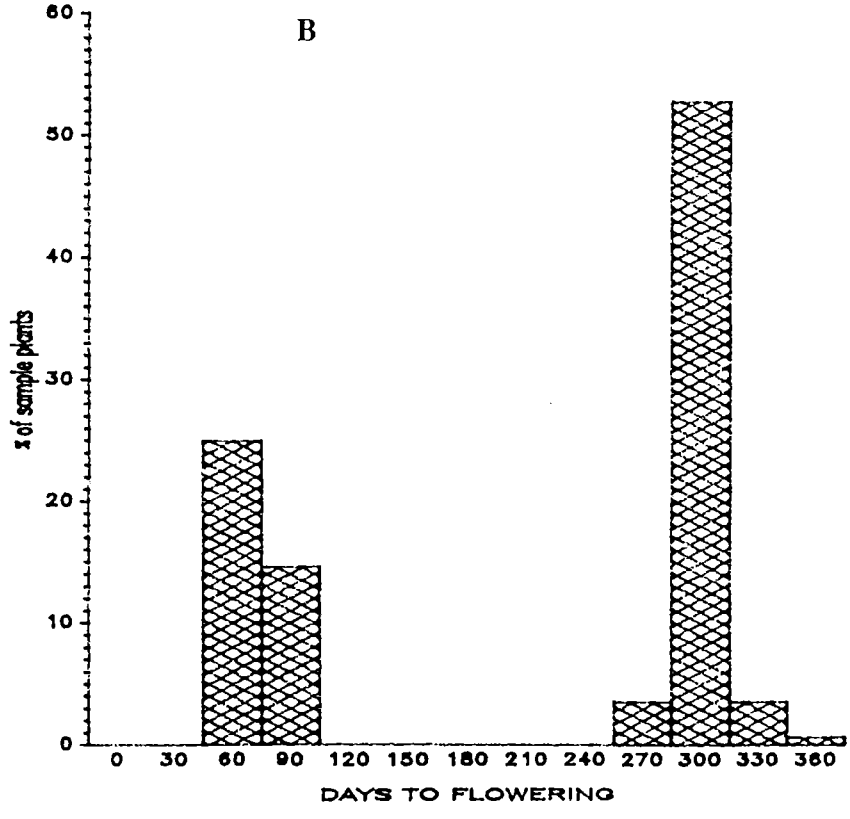


Figure 4.11. Frequency distributions of (A) Flower colour, and (B) days to flowering, in 1
sample Russell lupin plants in the germplasm collection.

Plate 4.7 In some plants flowering stopped soon after mid-March. On these plants new leaves emerged to occupy the sites of reproductive buds. (Picture taken 30 March 1988).

collection, including those that flowered in the autumn, flowered in spring (Plate 4.8). On average, genotypes HN, BN and CN had produced their first flowers much earlier than LD, QR, GR and EG (Table 4.4).

Dry matter production

The seasonal and annual dry matter production of individual plants is shown in Figure 4.12, 4.13a,b. Most of the annual dry matter yield was spring regrowth, and production of up to 750 g DM plant^{-1} was recorded from the two harvests. The frequency distribution of annual production showed that the modal class, about 30% of the plants, was 250-350 g DM plant^{-1}, with about 75% of plants falling in the range of 150-450 g DM plant^{-1} (Figure 4.12). Although up to 150 g DM plant^{-1} was harvested from 5 months old plants, about 80% of the plants produced only 13 - 63 g DM plant^{-1} by the end of autumn (Figure 4.13a). However, about 70% of the plants produced 175 - 375 g DM plant^{-1} during the spirng regrowth period (Figure 4.13b). While accessions HN, CN, NG, FL, RN and ON gave significantly higher dry matter yield in autumn than IR, SR, LD and GR; significantly higher spring regrowth yield was produced by ON, NG, HN and EG compared with IR, BN, SR and KR (Table 4.4).

Acceptability to sheep

Observations on the acceptability of the accessions to grazing sheep showed that the lupins were initially less preferred than other legumes and grasses growing in between the Russell lupin plots and plants. By the end of the experimental period, however, all the plants used for the grazing observation had been consumed by sheep (Plate 4.7). Nevertheless, there was a marked selective defoliation by sheep of plant parts. During grazing, the sheep generally consumed, in order of, flower heads, leaves and stems. Pods were generally rejected by the animals and the residue was primarily composed of pods (Plate 4.8). Even among the lupins, there was preferential grazing by sheep of some individual lupin plants before others.

Plate 4.8a Commencement of spring flowering in spring regrowth. Picture taken 13 October 1988. Note the remarkable regrowth and performance of Russell lupin genotypes grown in dry to very dry conditions.

Plate 4.8b One month later (10 November 1988), almost all plants in the collection including those that flowered in autumn, had flowered.

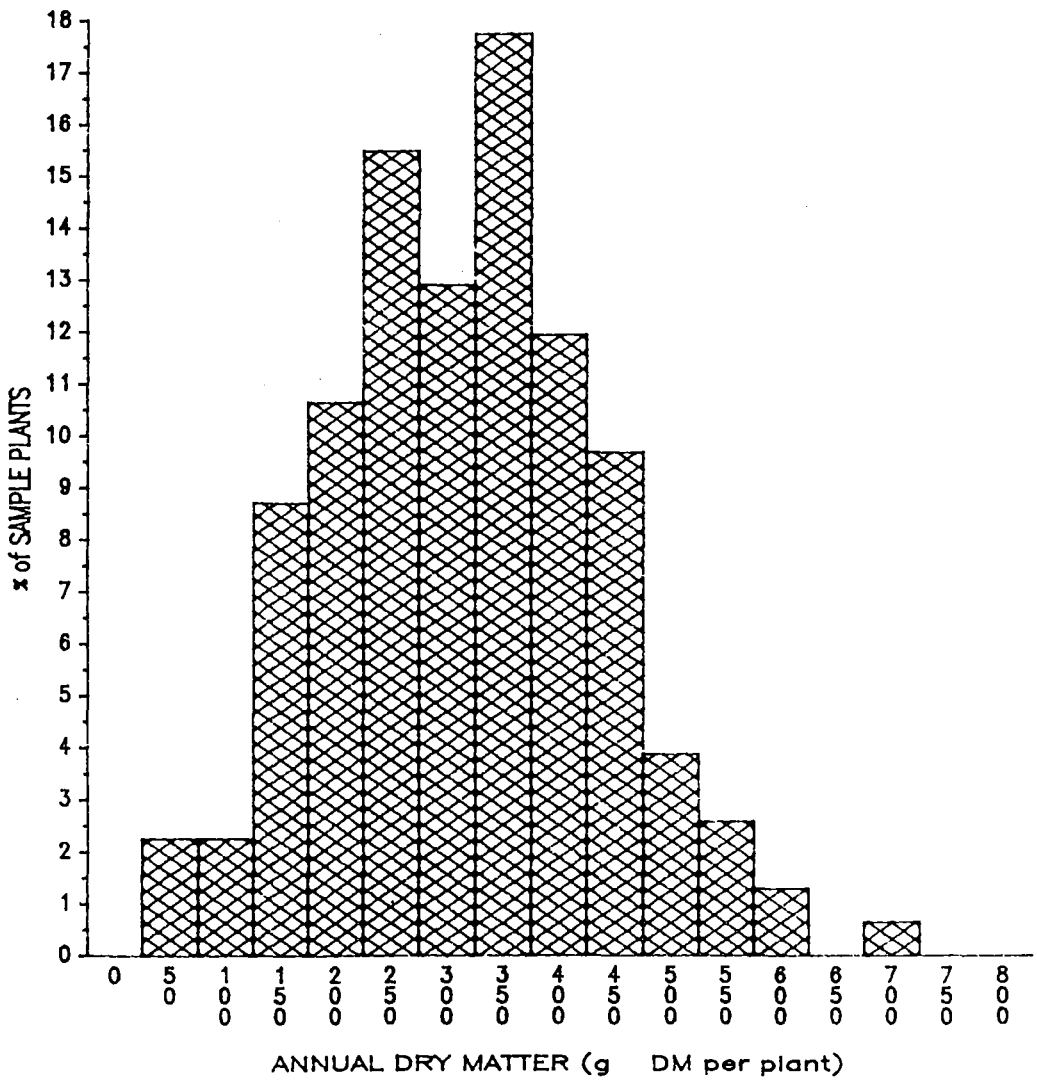
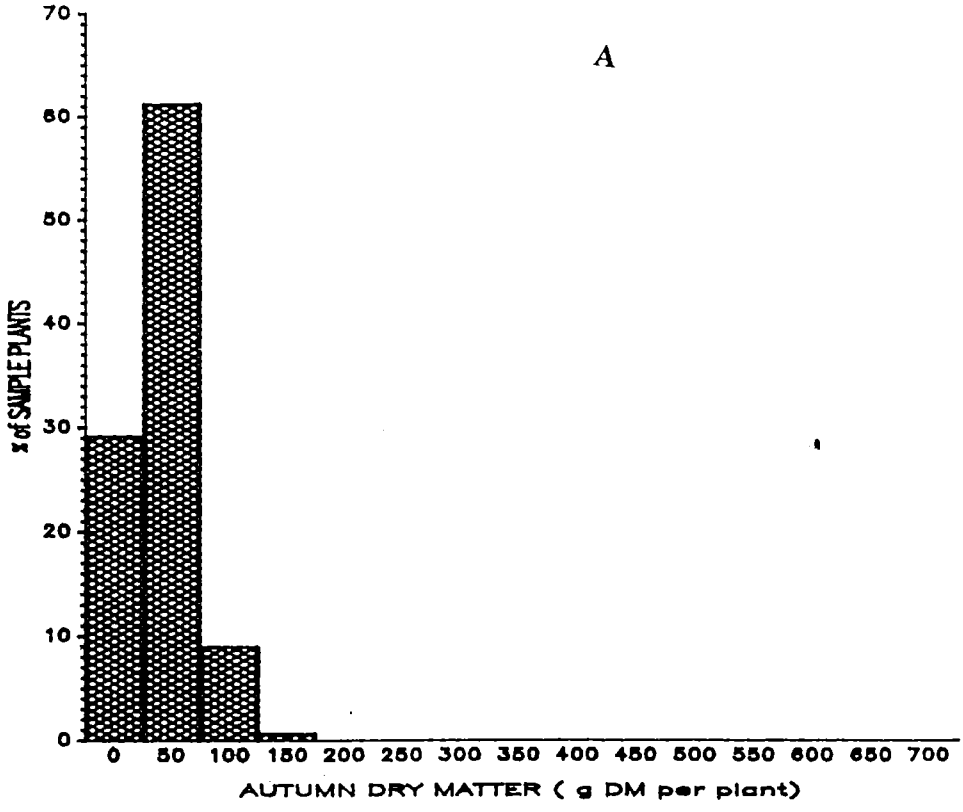


Figure 4.12. Frequency distributions of annual dry matter yield in 312 sample Russell lupin plants in the germplasm collection.



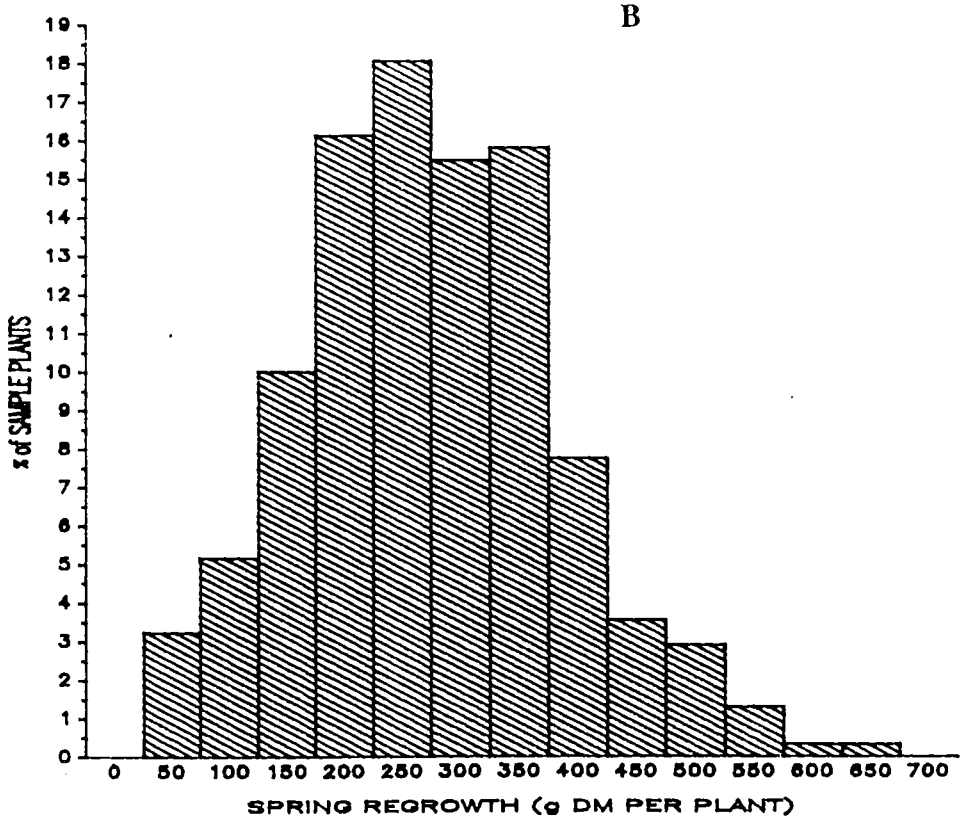


Figure 4.13. Frequency distributions of (A) early autumn, and (B) spring regrowth dry matter yield in 312 sample Russell lupin plants in the germplasm collection.

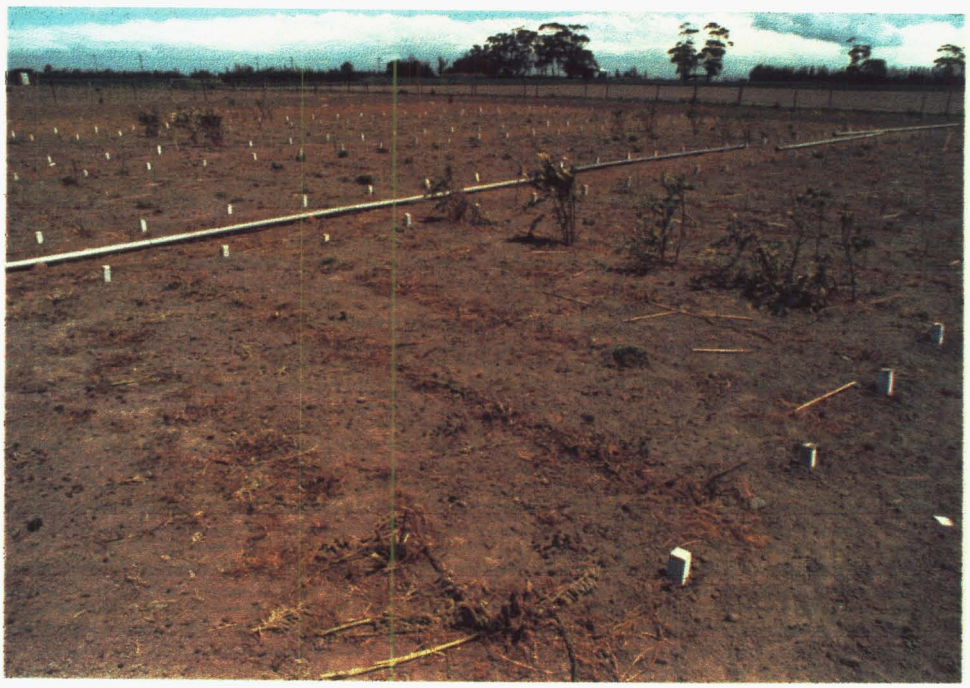


Plate 4.9 At the end of the experimental period, all the plants used for the grazing observation were eaten by sheep. The untouched plants are tree lupins (*L. arboreus*), mistakenly labelled as an accession of Russell lupin. (Measurements on tree lupins were not included in this study).

Plate 4.10 Pods were generally rejected by sheep and the Russell lupin residue was primarily pods.

4.3.3 Correlation among plant characteristics

The partial correlation coefficients of the variables included in the canonical variate analysis are shown in Table 4.5. Flowering in autumn and plant height in early-autumn were positively correlated to each other, but were negatively correlated with days to first flowering.

The correlation among the rosette leaf dimension variables in early-autumn (leaf diameter, leaflet length and leaflet width) was positive. More over, dry matter yield in autumn was positively correlated with plant spread in early-autumn.

Plant height in spring, plant spread in spring and dry matter yield in spring were positively correlated to each other. Plant height in spring was also positively correlated with raceme length.

4.3.4 Characterization of a Russell lupin collection

I. Canonical variate analysis

The probability level for the null hypothesis that all the canonical correlation are zero in the population suggested that, the first six canonical correlations are significant and worthy of consideration. Therefore, the first six canonical variate means, which accounted for 87.7% of the between accessions variation, can be plotted to show the separation of the 18 accessions.

The first canonical variate accounted for 35.2% of the between-accession variation (Table 4.6). A further 16.4% and 13% of the between-accession variation were explained by the second and third canonical variates respectively (Table 4.6). The other three canonical variates accounted for another 23.2% of the between-accession variation (Table 4.6).

Table 4.6 presents the standardised canonical coefficients. From the standardised loadings to be applied to each variable clearly indicated that the first canonical component

Table 4.5 Partial correlation coefficients of plant characteristics of a Russell lupin collection.

	Plant Spread[1]	Plant Height[1]	Leaf Diameter[1]	Leaflet Length[1]	Leaflet Width[1]	Leaflet Number[1]	Autumn DM[1]	Flowering in Autumn[2]	Raceme Length	Stem Width	Plant Spread[3]	Plant Height[3]	Spring DM[3]
Spread[1]	1.00												
Height[1]	0.25	1.00											
Leaf diam.[1]	0.28	-0.23	1.00										
Leaflet[1]													
Length	0.34	-0.15	**0.90**	1.00									
Width	0.18	0.03	**0.51**	**0.47**	1.00								
Number	0.03	-0.23	0.29	0.28	0.14	1.00							
Autumn DM[1]	**0.47**	0.22	0.16	0.17	0.28	-0.02	1.00						
Flowering in autumn[2]	0.14	**0.67**	-0.22	-0.18	0.03	-0.18	0.11	1.00					
Raceme leng.	-0.09	-0.01	0.08	0.05	0.16	-0.00	0.09	0.03	1.00				
Stem Width	0.03	0.02	0.07	0.06	0.11	0.04	0.06	0.03	0.26	1.00			
Spread[3]	0.38	0.05	0.25	0.27	0.23	0.13	0.33	0.00	0.23	0.23	1.00		
Height[3]	0.19	0.04	0.22	0.23	0.22	0.07	0.21	0.00	**0.60**	0.33	**0.47**	1.00	
Spring DM[3]	0.35	0.02	0.26	0.25	0.17	0.08	0.33	-0.08	0.13	0.19	**0.58**	**0.49**	1.00
Days to first Flowering	-0.15	**-0.69**	0.24	0.20	-0.03	0.20	-0.12	**-0.99**	-0.05	-0.04	-0.01	0.01	0.07

[1]- Measurements made in early autumn;
[2]- Tendency of first flowering before exposure to cool conditions &/or short days;
[3]- Measurements made in late spring.

Table 4.6 Standardised canonical coefficients for canonical variables and the percentage of between-line variation accounted for by the first six canonical variates.

	CANONICAL VARIATE					
	1	2	3	4	5	6
AUTUMN						
Plant spread	-0.3622	-0.1758	0.4815	-0.0358	-0.5167	0.1340
Plant height	-0.1406	0.3082	-0.5459	-0.5750	0.3631	0.0617
Leaf diameter	0.0560	0.0223	-0.0539	-0.5823	-0.3522	**-0.6840**
Leaflet leng.	**-0.6296**	-0.2408	-0.3762	0.1106	**1.0058**	**0.7193**
Leaflet width	0.0429	0.0853	0.5371	-0.1880	-0.1372	0.4338
Leaflet numb.	-0.2181	0.2127	-0.1356	0.4107	-0.2222	**0.7717**
Dry matter	**0.6027**	0.4602	-0.2653	0.2535	0.4249	0.2477
Flowering in Autumn	**1.1291**	-0.2890	**2.1403**	**1.7692**	**3.9112**	-0.1553
SPRING						
Plant spread	0.0731	-0.0479	0.5364	-0.2858	-0.3584	-0.2146
Plant height	-0.4726	**1.1670**	-0.1825	-0.3542	-0.0707	**-0.5936**
Dry matter	0.2705	0.0612	-0.1471	0.7148	-0.0605	0.0554
Raceme length	-0.2162	-0.4530	-0.1523	0.6194	0.1537	0.1839
Stem width	0.2854	-0.0354	0.5505	-0.1564	0.4699	0.0339
Days to first flowering	**0.6626**	-0.2047	**2.2343**	**1.7531**	**4.2617**	-0.4845
Percentage variation accounted for	35.2	16.4	13.0	9.0	8.8	5.4

was a weighted difference of leaflet length (-0.6296) and autumn flowering (1.1291), days to first flowering (0.6626) and autumn dry matter (0.6027). The second canonical component explained plant height in spring (1.1670) as an important variable in separating the 18 accessions of Russell lupin. Both the third and the fourth canonical components revealed that autumn flowering and days to first flowering had the greatest discriminatory effect. The fifth canonical component also revealed that days to first flowering (4.2617), autumn flowering (3.9112), and leaflet length (1.0058) play a much larger part in separating the different accessions of Russell lupin. The sixth canonical component contained a large contribution from leaflet number (0.7717), leaflet length (0.7193), leaf diameter (-0.6840), and plant spread in spring (-0.5936).

Although the suggested dimensionality of the vectors required to represent the relationship between the different accessions was six, plotting all pairs of the first six canonical variates was not easy. Despite this, Figure 4.14 shows a three-dimensional scatterplot of the 18 accessions of Russell lupin. The scatter of the points in Figure 4.14 indicates that there is definite separation among the different Russell lupin genotypes, as HN, RN, BN, CN and FL , which all lie to the left of the Figure, stand out as a single group. However, there is little evidence of any clear cut separation among the accessions based on the third canonical component, except possibly for HN and FL which lie at the extremes. Therefore, as the information for separating the 18 accessions seems to be mainly contained in the first two canonical variates, any obvious variation trends can be displayed with the plane defined by a two-dimensional scatterplot (Figure 4.15).

II. Trends in variation

The 95% confidence circles were drawn around the point representing each accession in the plane defined by the first two canonical components (Figure 4.16). This plot dissects the collection into three distinct groups of accessions. It suggests that Connie lupin, the control accession in this study, was most similar in performance to RN, BN (New Zealand), and FL (Portugal); but very different from ON (New Zealand), and nearly all the accessions obtained from the northern European countries. More over, HN from

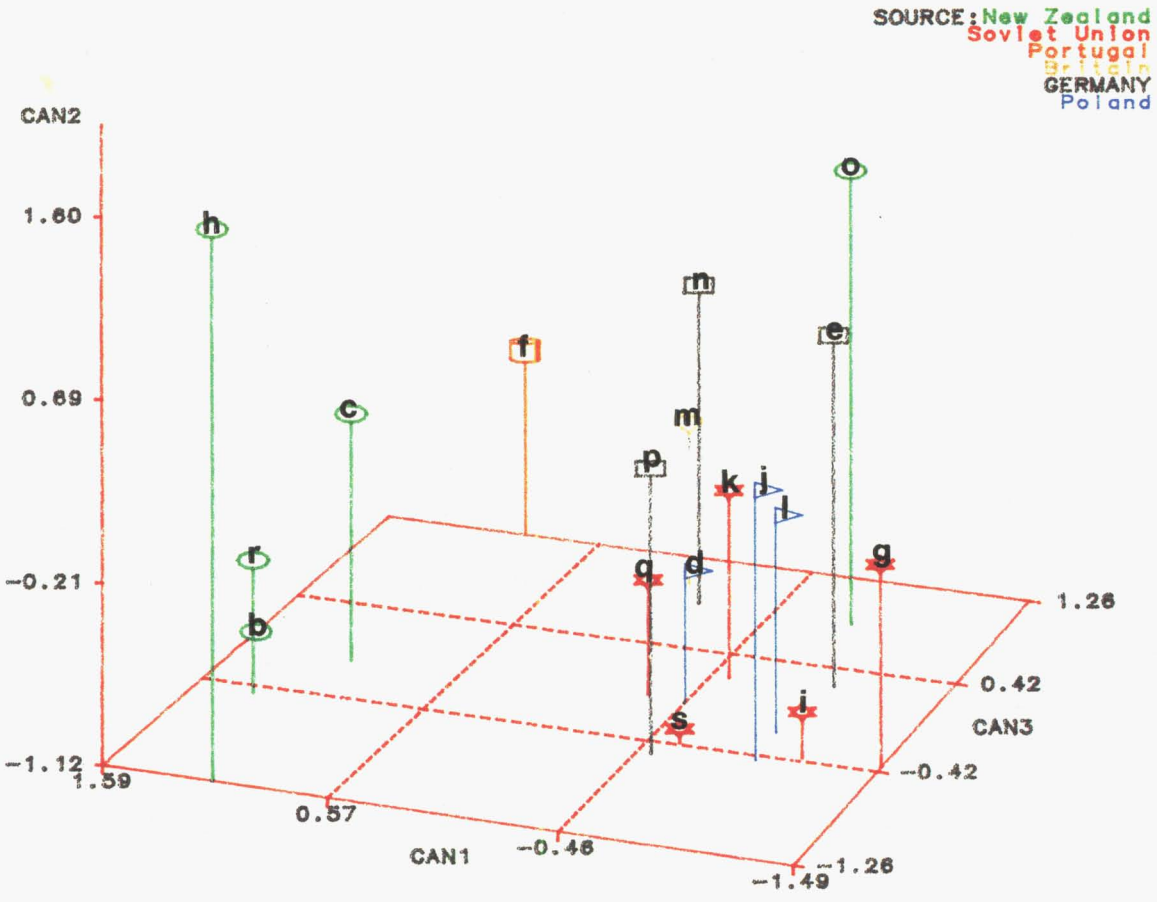


Figure 4.14 Canonical variate analysis: Three-dimensional scatterplot of 18 Russell lupin accessions. Co-ordinate axes correspond to the first three canonical variate means calculated from the plant characteristics included in the analysis. The symbols refer accession codes given in Table 4.1.

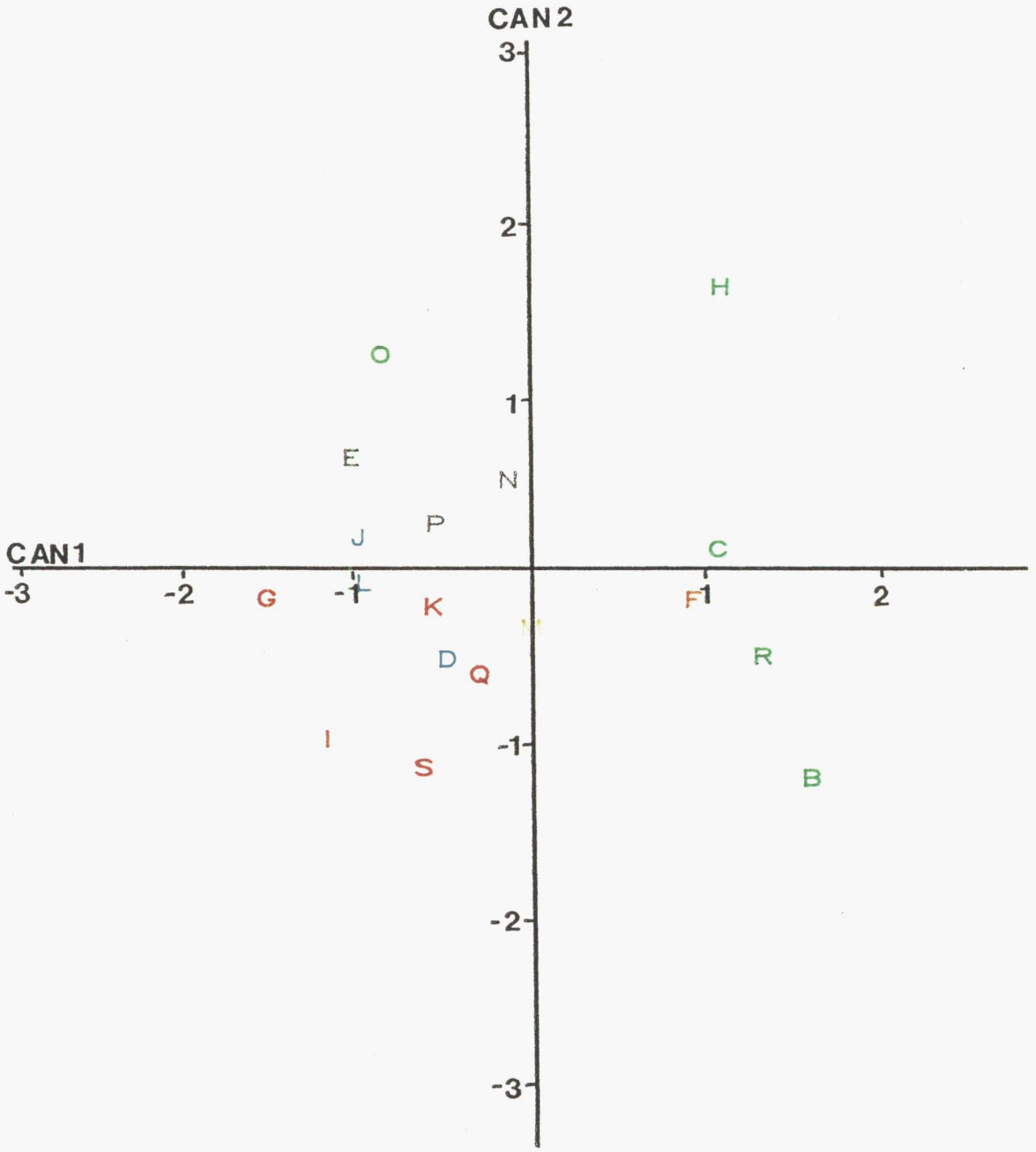


Figure 4.15 Graph of the canonical variate analysis showing Russell lupin accession relations on the first two canonical axes. The symbols refer accession codes given in Table and colours indicate seed sources: Green (New Zealand), Red (USSR), F (Poland), Black (Germany), Orange (Portugal), and Gold (UK).

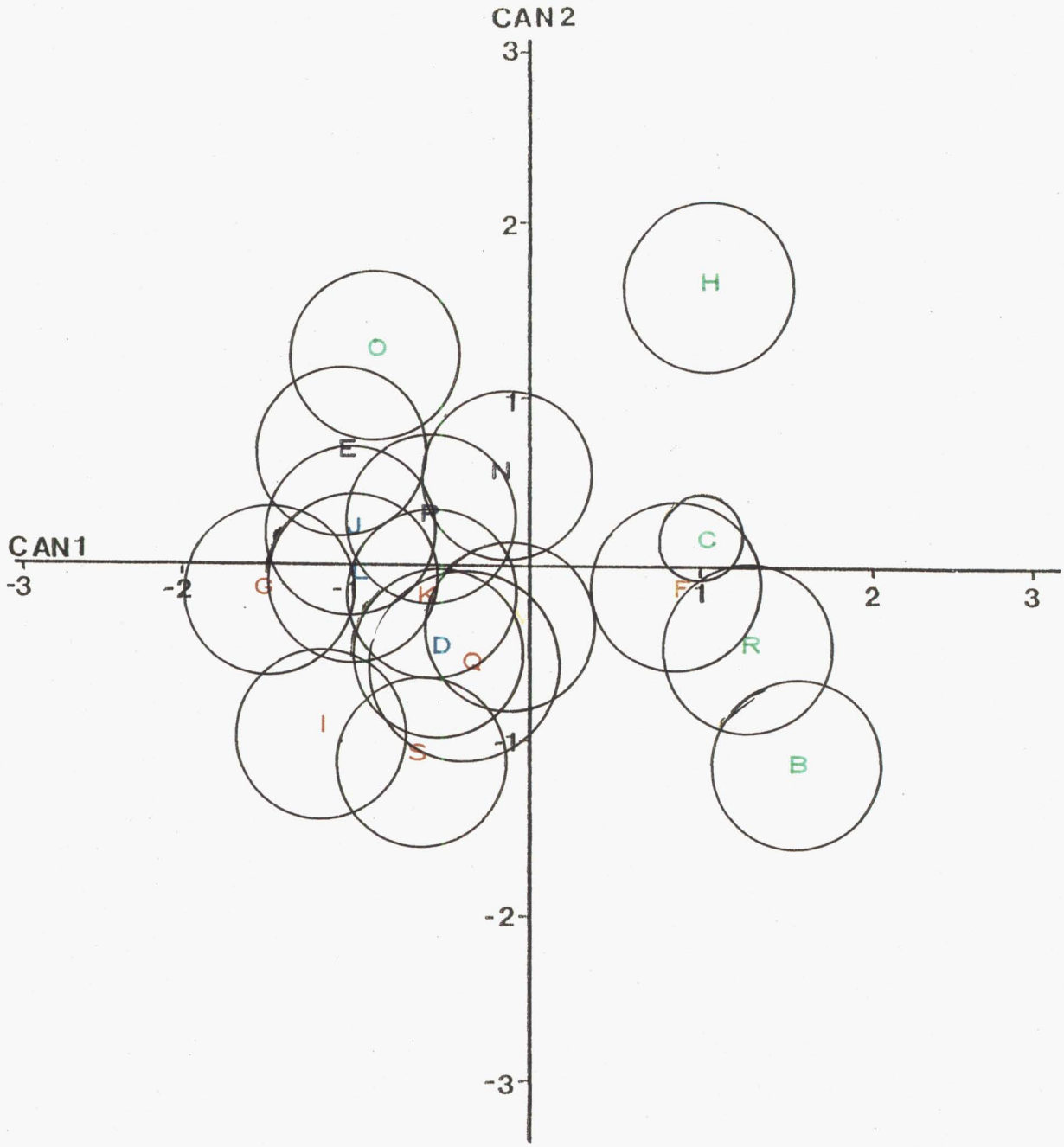


Figure 4.16 Graph of the canonical variate analysis showing accession relationships on the first two canonical axes. The circles round the point representing each accession indicate the 95% confidence regions. The symbols refer accession codes given in Table 4.1.

New Zealand seems to stand out from the rest of the Russell lupin collection and stands by itself.

Axes illustrating the trends in variation of the plant characters that had the greatest discriminatory effect were drawn (Figure 4.17). These axes were not computed but were used as aids to interpretation. The similarities in the trends depicted by the axes for these characters were a reflection of the relatively large correlation coefficients between these characters (Table 4.5).

The relationships shown in figure 4.17 have been illustrated schematically with the days to first flowering, autumn flowering, autumn dry matter yield and leaflet length axes combined into one axis-flowering response (Figure 4.18). Moving from the origin along this axis to the right of the Figures 4.15, 4.16, or 4.17, the accessions were early flowering, had a tendency to produce their first flower before exposure to cold and/or short days and were high yielding in autumn although they tended to have smaller leaflets. These were considered largely as non-vernalization requiring types. In the opposite direction towards the left in Figures 4.15, 4.16, or 4.17, the accessions were late flowering, produced their first flower only after exposure to cold and/or short days, and had very low autumn dry matter production although they had longer leaflets. These were considered largely as vernalization requiring types.

The axes depicted for the second canonical component essentially weighted size and yield variation and were combined into one axis-aerial biomass production- and approximately subtended a right angle with the flowering response axis (Figure 4.18). Thus accessions situated towards the top in Figures 4.15, 4.16, or 4.17 were tall plants, with a high horizontal spread at maturity, and gave a higher dry matter yield in spring. These produced high aerial biomass. Those genotypes located towards the bottom in Figures 4.15, 4.16, or 4.17 were short plants, with low horizontal spread at maturity, and very low spring dry matter yield, indicating low aerial biomass production.

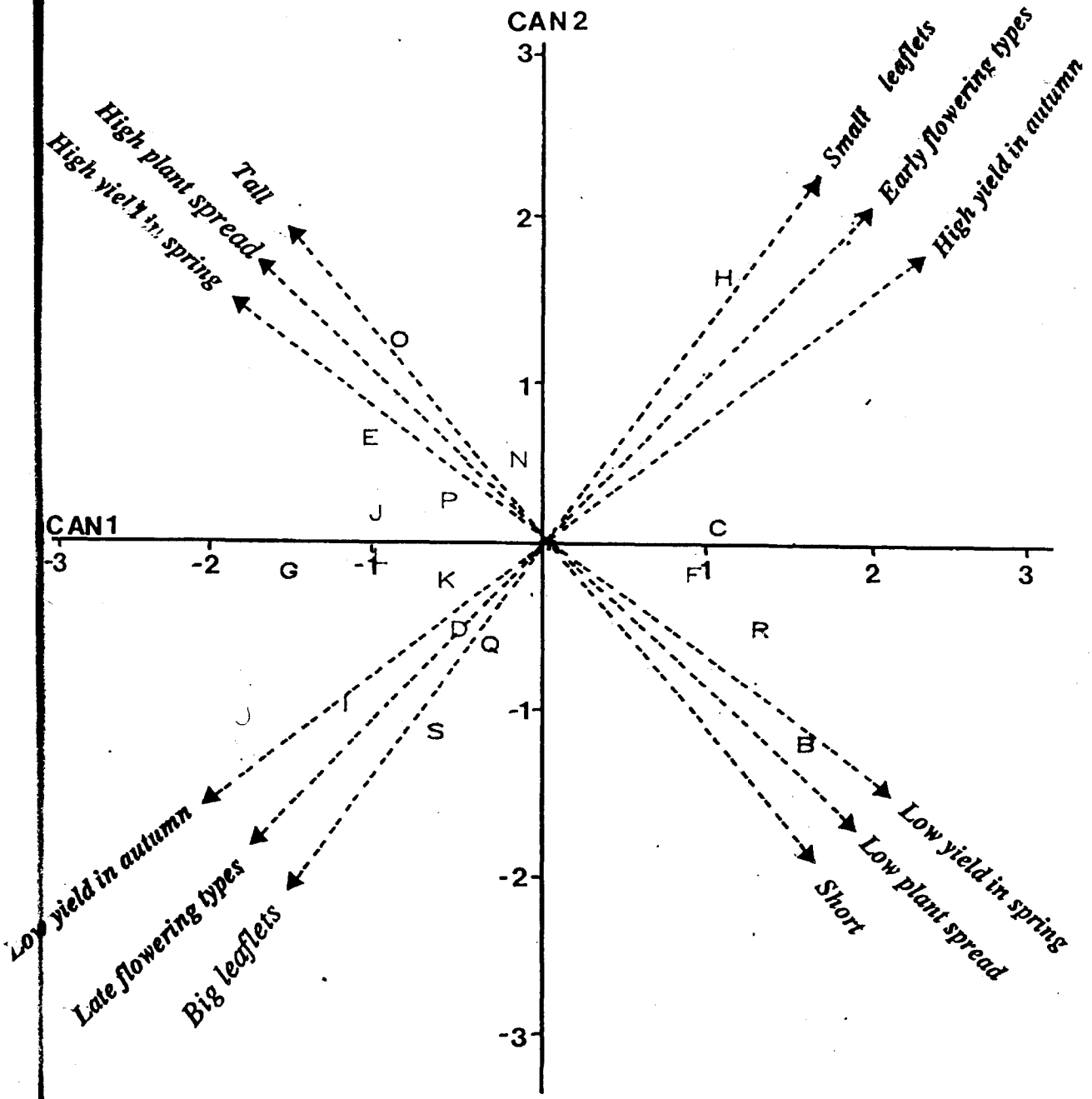


Figure 4.17 Graph of the canonical variate analysis showing accession relationships and axes (broken lines) illustrating the trends in variation of the plant characteristics that had the greatest discriminatory effect. The symbols refer Russell lupin accession codes given in Table 4.1.

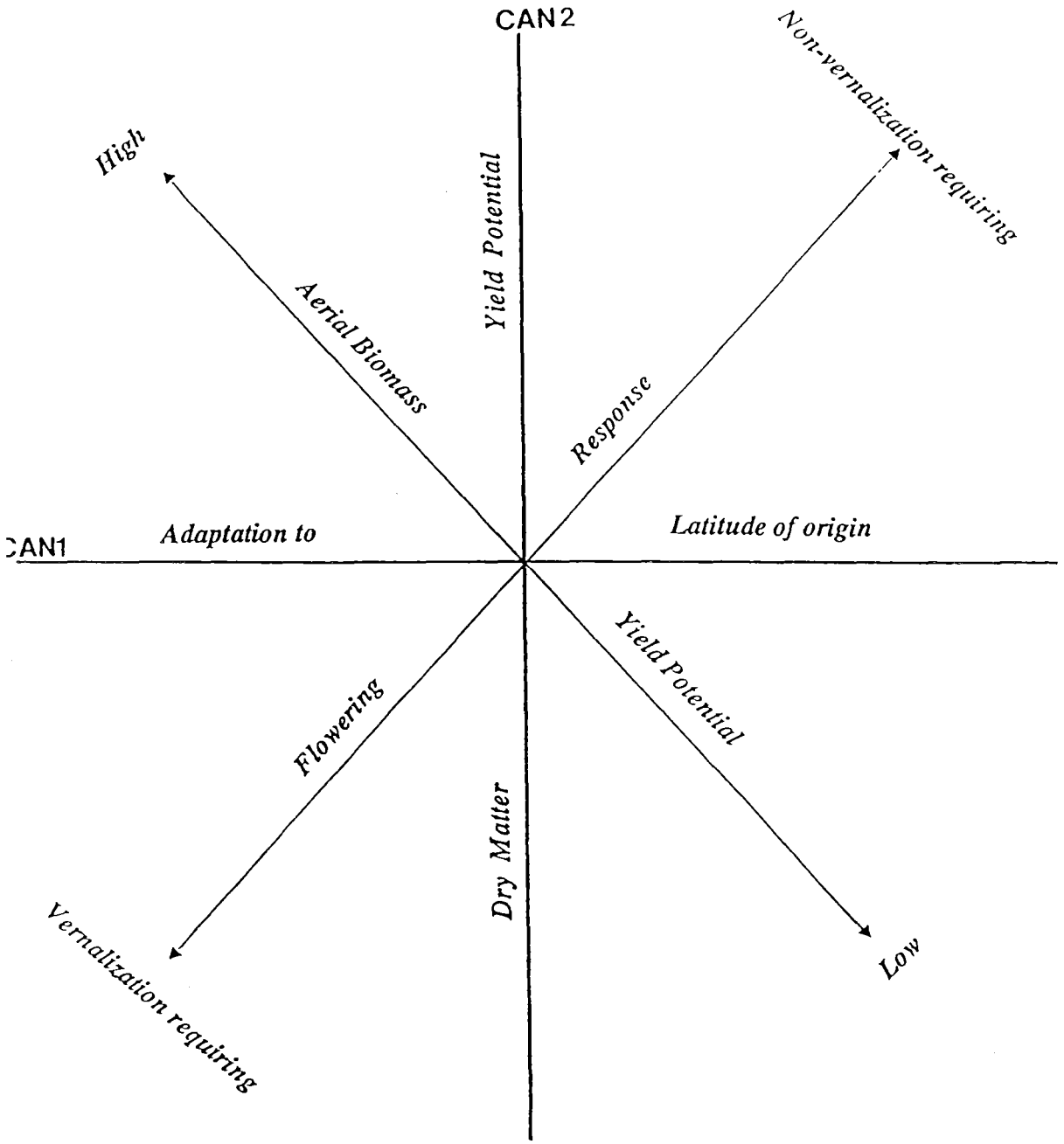


Figure 4.18 Schematic representation of the plant characteristics that had the greatest discriminatory effect in separating 18 Russell lupin accessions on the plane defined by the first two canonical variates.

4.4 DISCUSSION

4.4.1 Grouping in the Russell lupin germplasm collection

This study on the New Zealand germplasm collection of Russell lupin has shown that there is between and within accession variability in morphology, growth, performance and flowering characteristics. The between accession variability existed at both a univariate and a multivariate statistically significant level. However, the identification of a clustering of groups of Russell lupin accessions by any single plant characteristic was not consistent throughout for most of the plant characteristics considered in this study (Tables 4.3, 4.4). Obviously Analysis of Variance was a less appropriate method of germplasm characterization when several correlated plant characteristics were measured. On the other hand, canonical variate analysis proved to be a useful statistical method for the characterization of the genetic variability among the Russell lupin accessions.

The first three canonical variates explained 64.6% of between-accession variation. Nevertheless, close examination of the scatterplots in Figures 4.14 and 4.15 indicates that two dominant canonical variates separated the accessions. The major discrimination was observed on the first canonical axis. By inference from these two axes, the first canonical variate measured adaptation to latitude of origin. Clearly accessions which lie to the right in Figures 4.15, 4.16, or 4.17 were adapted to lower latitudes, produced their first flower before winter and/or short days and were early flowering. This group composed genotypes from New Zealand (HN, CN, RN and BN) and Portugal (FL). Conversely those accessions situated to the left in Figures 4.15, 4.16, or 4.17 were adapted to high latitudes, only flowered after exposure to cold and/or short days and were late flowering. This group comprised the accession ON (New Zealand) and all genotypes from north European countries. New Zealand and Portugal are located at a latitude of 35 - 44^{o} from the equator, whereas Britain, Poland, Germany, and the USSR are located at above latitude 47^{o}N.

Flower initiation of legumes is controlled by three complementary processes:- vernalization, a long day requirement and a high temperature requirement (Gladstones and Hill, 1969; Rahman and Gladstones, 1972; 1974; Thomas, 1980). There is a species and

varietal adaptation to this situation. In both field (Gladstones and Hill, 1969; Rahman and Gladstones, 1974) and controlled environment studies (Rahman and Gladstones, 1972), flower initiation of *L. angustifolius* was controlled mainly by its vernalization requirement. *Lupinus cosentinii* had a lower vernalization requirement than *L. angustifolius* and initiation was substantially accelerated by long photoperiod and high temperature post-vernalization. However, *L. luteus* showed the greatest response to photoperiod among species tested in a controlled environment study (Rahman and Gladstones, 1972). There is no published work on the flowering response of Russell lupins and even from this study, it is difficult to speculate on the vernalization or photoperiod requirement of the Russell lupin collection. What seems certain however, is the non vernalization requirement of all, but one, of the New Zealand accessions (HN, CN, RN and BN) and the accession from Portugal (FL). The flowering response of these accessions could be attributed to the milder winter conditions in both New Zealand and Portugal than in the north European countries from which most of the other Russell lupin genotypes were obtained. However, the association of ON, from New Zealand, with all accessions obtained from the higher latitude countries was an exception.

The relationship between flowering response and the latitude of origin seen in the accessions in this study is complemented by a study at Palmerston North on the flowering responses of different white clover cultivars and lines with their latitude of origin (Thomas, 1980). On the basis of latitude of origin and flowering response, white clover plants were grouped into two groups. Mediterranean types with all originated at low latitudes, and summer-growing, high latitude types. Flower initiation in Mediterranean types in response to cool conditions started in autumn and continued through the winter. Onset of initiation was earliest in plants from lowest latitudes. High-latitude ecotypes did not begin flower initiation in response to cool condition until after June or July, or in some individual plants, October (Thomas, 1980).

The second canonical component in the present study reflected plant size and spring yield characters to have the most discriminatory effect. As the annual dry matter production of Russell lupin plants in this study was mainly dominated by spring dry matter yield (Table 4.4, Figure 4.12, 4.13), the significance of the second canonical component could be considered to be as potential total dry matter yield. Thus on differing

planes of the Figures 4.15, 4.16, or 4.17, accessions ON, HN (New Zealand), EG and NG (Germany), had greater total dry matter production than BN (New Zealand), SR and IR (USSR).

The 95% confidence circles shown for each accession in Figure 4.16 define the limits of uncertainty of each accession in the canonical analysis, i.e., the probability is 5% that an accession will lie outside its confidence circle. Therefore, Connie lupin clearly required no vernalization compared with the population mean of all 18 accessions. This may be the function of its adaptation to the less severe climate in New Zealand than in the northern European countries, from which most of the other accessions were obtained. The 95% confidence circle approach produced three distinct groups of accessions (Figure 4.16). Combining this information from the 95% confidence circles with the interpretation of the canonical axes gives four distinct groups of Russell lupin accessions. The characterstics of these groups are shown in Table 4.7.

It is hypothesised that the morphological, performance and growth variability in Russell lupin observed here may have been due to at least three contributing factors. Firstly, with their hybrid nature it is possible that Russell lupins do not breed true from seed. This suggests that the within accession variability observed in this study is likely to occur. Another factor contributing to the variability may have been due to the unselected nature of the seed accessions used in this study. The populations derived from the unselected seed accessions showed diverse natural variability, and this may have depended on the extent of sampling during plant/seed collection. This may further contribute to the within and among accession variability observed in the Russell lupin genotypes.

The final hypothesis is related to the grouping of Russell lupin accessions with respect to their latitude of origin in the germplasm collection. Although the Russell lupin was originally bred and developed in the United Kingdom, its accidental and/or deliberate introductions to countries such as Portugal and New Zealand may have led to the formation of new gene combinations that have increased the plant's adaptability to the new environment.

Table 4.7 Proposed grouping of Russell lupin genotypes and their main characteristics as identified by canonical variate analysis.

Group	Accessions	Plant characterstics
I	CN, FL, RN and BN	-Low latitude type -Non-vernalization requiring -Early flowering -Small plants with -Medium to small leaflets -Medium to short -Medium to low DM yield -Medium to low spread -Medium to short racemes
II	HN	-Low latitude type -Non-vernalization requiring -Early flowering type -Large plants with -Small and narrow leaflets -Tall -High DM yield -High spread -Long racemes
III	ON, EG, NG, PG, and JD	-High latitude type -Vernalization requiring -Late flowering -Large plants with -Long and wide leaflets -Medium to tall -Medium to high DM yield -Medium to high spread -Medium to long racemes
IV	DD, GR, IR, KR, LD, MU, QR and SR	-High latitude type -Vernalization requiring -Late flowering -Small plants with -Long and wide leaflets -Medium to short -Medium to low DM yield -Medium to low spread -Medium to short racemes

Accession refers to accession codes given in Table 4.1.

4.4.2 Plant selection and germplasm characterization

Partial correlation of measured characters showed that flowering in autumn and plant height in early-autumn were positively correlated with each other, but were negatively correlated with days to first flower. This meant that plants which tended to produce their first flower before exposure to cold and/or short days reached their reproductive phase earlier and initiated flower buds in early-autumn. The relationship between stem initiation and elongation with flowering indicates that autumn-flowering, plant height in early-autumn and days to flowering are all inter-related.

Tall plants with a high plant spread also produced high dry matter yield in both autumn and spring (Table 4.5). This suggests the possibility of selecting a cultivar of Russell lupin with high plant spread, i.e. a plant with increased leaf mass. The relative proportions in the total dry matter yield of leaf, stem, flowers and pods were not measured in this study. However, pods were not eaten by grazing sheep. Further, as the nutritional quality of a *L. angustifolius* plant components have shown significant differences (Burt, 1981), it is important to measure the nutritional quality of the Russell lupin plant parts as well as to measure their contribution to the total dry matter yield. Such work was beyond the scope of this thesis.

Despite the rejection of pods by grazing sheep, this work has shown that Russell lupins will be grazed up to flowering. The alkaloid content of the Russell lupin genotypes tended to be lower in spring than in autumn (Gibbs, 1988). A greater number of the genotypes contained a 1.50 to 1.99% alkaloid in spring, compared to 2.00 to 2.99% alkaloid in autumn (alkaloid concentration was expressed as a percentage of the dry matter). Chemical analysis of the variation in alkaloid content of Russell lupin genotypes was based on bulked leaf material from five sample plants. This limits any likely screening and identification of individual plants suitable for further development as a low alkaloid Russell lupin. Further, the noted preference of sheep to some individual plants before others suggests that selection of individual Russell lupin plants from the collection might further improve the acceptability of Russell lupins after flowering. Therefore, future study on the variation in alkaloid content of Russell lupin genotypes should be based on the study of individual plants over the different growth stages.

The positive correlation among the leaf dimension variables in early-autumn indicated that plants with long leaflets also had wide leaflets. However, both these characters were independent of both autumn and spring dry matter production, and thus indicate little hope of plant improvement through selection for leaf size.

However, the relatively longer and wider leaflets of rosette leaves compared with stem leaves suggested that characterization of Russell lupin accessions according to their leaf dimensions will change during the growing season and/or depends on the sampling procedure used. This demonstrated the possible limitation of too rigid use of this character during characterization.

A reasonably accurate representation of the relations betweeen the 18 genotypes was obtained by plotting their canonical variate means. In spite of the within variability observed in this study, plotting of the canonical variate means of accesssions have not clearly shown the pattern of the within variability. This may be a major criticism of employing canonical variate means in the analysis. However, Thomson (1974) in Britain studied the winter hardiness components in 24 rye grass varieties. He also applied the canonical variate means to represent the relationship between the 24 varieties. It was possible to characterize varieties with adaptation to winter conditions and spring yield as discriminating attributes.

Throughout this study, conditions were dry to very dry. As a result plants may have had a restricted root system and reduced soil available water. In spite of this, the remarkable performance and regrowth of Russell lupin (Plate 4.6), in these climatic conditions demonstrated the plant's ability to withstand drought. This may have been due to a deep root system that can extract available soil moisture from deep in the profile.

Most of the characters measured in this study were environmentally influenced. The exceptions were plant habit and flower colour. Thus, the performance of the Russell lupin genotypes depended on both genetic and environmental factors. Because the characterization was conducted at one site, where there was drought and a mild winter, it may have discriminated against accessions from high latitudes. However, the main aim of

this study was to observe and classify the collection so the data obtained would be used by other researchers, in particular the grouping of like accessions would allow a reduction of the number of genotypes being used to manageable proportions. Thus, from this work it should be possible to evaluate a smaller number of Russell lupin genotypes in the hill and high country for use in that environment.

4.5 CONCLUSIONS

In terms of overall relationships, canonical variate analysis identified four distinct groups of accessions in the collection. Characterization of the genotypes using univariate statistics, or by simple visual observation was extremely difficult.

A latitude of origin pattern of variation was represented along the first canonical axis, which accounted for 35.2% of the between-accession variation. Along this axis, all, but one, accessions from New Zealand and the accession from Portugal were grouped together as opposed to the group of accessions from northern Europe. Flowering before exposure to cold and/or short days, days to first flower, dry matter yield in autumn and leaflet dimension appear to be the most discriminatory attributes in separating accessions along the first canonical axis.

Along the second canonical axis, which accounted for a further 16.4% of the between-accession variation, there was wide variation in plant size and spring dry matter yield potential. Two New Zealand accessions (HN & ON), with a higher yield potential than the mean of the 18 accessions were identified, although these accessions show different flowering responses, perhaps, to cool conditions along the first canonical axis. Conversely, all accessions from the USSR were vernalization requiring, high latitude types, and had a low dry matter yield in both autumn and spring.

Connie lupin (CN), used as the control, was an early flowering, non-vernalization requiring, low latitude type, and was of medium plant height and had medium dry matter yield potential.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSION

5.1 Introduction

Work by Scott and Covacevich (1987) has shown that the Russell lupin has the agronomic potential to become a grazing legume, adapted to New Zealand hill and high country soils and climate. The present study has provided information on its morphology, growth, performance, seedling emergence, and the characterization of the New Zealand germplasm collection.

5.2 Seedling emergence studies

Lack of adequate information on methods of establishment of alternative pasture species is a major problem in pastoral agriculture. The problem is further aggravated when germplasm collections of potential species are assembled for plant introduction purposes. Thus an initial assessment of factors affecting seedling emergence and establishment is indispensable if satisfactory establishment methods are to be designed for future research and/or large scale production of alternative pasture species.

As in many other legume species, a high proportion of freshly (hand) harvested Russell lupin seeds are hard. However, the evidence in this study and from work on other legumes (Porter, 1949; Quinlivan, 1971; Horn and Hill, 1974; Rolston, 1978; Maguire, 1980; Liu, *et al.*, 1981; Voon, 1986) indicates that scarification with concentrated sulphuric acid can be used to improve germination of hard seed. Scarification by immersion in concentrated sulphuric acid (36N), for 30 to 45 minutes, produced more than 75% normal Russell lupin seedlings 15 days after sowing in a glasshouse (Chapter 3).

The results also show that emergence responses for depth of sowing and temperature can be quantitatively described in terms of the time taken to the onset of emergence (lag period), the time to achieve 50% emergence, the rate of emergence and the

maximum emergence attained in a specified test period. The emergence rate, the time taken to 50% emergence and the maximum emergence attained 57 days after sowing were significantly improved by sowing at 1 or 2 cm depth (Chapter 3). Moreover, seedling growth parameters (seedling height, root length, and oven dry weight of tops and roots) were also significantly improved by sowing at 1 or 2 cm below the soil surface. Although other herbage legumes have given maximum emergence within the range 1 - 3 cm (Janson and White, 1971; Peiffer *et al.*, 1972; Wan Mohamed, 1981; Williams, 1981; Voon, 1986), sowing at 3 cm or deeper did not give 50% emergence by 57 days after sowing, and were thus considered unsatisfactory depths for establishment of Russell lupins.

In controlled temperature cabinets where depth of sowing was held constant (1 cm), increasing the temperature from 10 to 25 ^{o}C decreased the lag period. The lag period was 8 days at 10 ^{o}C and fell to 2 - 3 days at 20 and 25 ^{o}C (Chapter 3). The time taken to 50% emergence in the Russell lupin was increased by low temperature, although differences among 15, 20, and 25 ^{o}C treatments for the speed of seedling emergence were relatively small. Furthermore, total emergence, 15 days after sowing, increased from 10 to 20 ^{o}C, but was decreased considerably at 25 ^{o}C. The finding that temperature in the range 10 to 20 ^{o}C does not limit the final total emergence of Russell lupin (Chapter 3) could be expected from previous work on lucerne (McWilliam *et al.*, 1970; Young *et al.*, 1970; Hampton *et al.*, 1987), white clover (McWilliam *et al.*, 1970; Hampton *et al.*, 1987), red clover (Hampton *et al.*, 1987), and subterranean clover (McWilliam *et al.*, 1970; Young *et al.*, 1970; Silsbury *et al.*, 1984; Hampton *et al.*, 1987). The present study also demonstrated that the critical germination and emergence temperature for Russell lupin is less than 10 ^{o}C. This indicates the feasibility of successful introduction of Russell lupin to New Zealand hill and high country where low temperatures of <5 ^{o}C, are regularly recorded. However, further research on low temperature effects on seedling emergence and establishment of Russell lupin is required, particularly in the field.

In these experiments, the conditions of scarification, depth of sowing and temperature were standardized as far as possible. In so doing an almost ideal environment for emergence and growth was created, i.e., the emerging seedling had ample water and good aeration. It may be argued that such an environment is rarely found in the field and this may be a possible cause of criticism for undertaking these studies in controlled

environments. Moreover, as the seedling emergence studies were carried out by using seeds of one of the accessions from New Zealand (Connie), extrapolation of the results, especially those for temperature, may not hold true for all of the accessions of Russell lupin collected from different countries. This is because in the characterization experiment, four quite distinct groups of accessions in the germplasm collection were identified (Chapter 4), which showed different responses related to their latitude of origin.

5.3 Characterization of a Russell lupin collection

Characterization of the genotypes in the collection using univariate statistical analysis (ANOVA) or visual observation was extremely difficult. However, the scatterplot of the canonical variate means of accessions showed a pattern of variation within the Russell lupin germplasm collection. Thus, canonical variate analysis proved a useful statistical method for grouping the accessions.

The first canonical variate accounted for 35.2% of the between accession variation and essentially measured a pattern of variation based on the latitude of origin. Days to first flowering, tendency to produce flowers before exposure to cold and/or short days, autumn dry matter yield, and leaflet dimensions had the greatest discriminatory effect. A further 16.4% of the between accession variation was accounted for by the second canonical axis which weighted plant size as the discriminatory attribute along the axis.

All, but one, New Zealand accessions and the accession from Portugal are low latitude, non-vernalization requiring, and early flowering. Of these, HN from New Zealand was tall with a higher yield than the mean of the 18 accessions. All the accessions from north European countries and one accession from New Zealand (ON) were high latitude, vernalization requiring, and late flowering. However, on the basis of plant size and dry matter yield, this latter group was categorized into a group consisting of tall plants, and high yield potential; and short plants and low yield potential. The former group comprised one accession from New Zealand (ON) and all the three accessions from Germany. The latter group was composed predominantly of all the accessions from the Soviet Union.

It is interesting that one accession from New Zealand (ON) behaved like the accessions from northern Europe. Further, two of the New Zealand accessions (HN & ON) had a higher yield than Connie (CN) and the mean of the 18 accessions. These two accessions have different flowering responses. They are already adapted to the New Zealand environment. The presence of such diversity in flowering response should provide good opportunity for further breeding of this plant to adapt it to the New Zealand environment.

It seems accessions from high latitudes would need to be sown in autum or early winter if they are to flower and produce a good seed set in the following summer. However, spring sowing of the same genotypes would increase quality forage production, at least in the first year, as the accessions would tend to remain vegetative until the following spring and this would produce more leaf.

The use of multivariate techniques in plant evaluation data have been clearly illustrated by Burt *et al* (1979), and Burt and Williams (1979). These workers used plant evaluation data as a continuous process-beginning with an ecological assessment at the point of collection, continuing through the quarantine period, and modified by studies of continuous variation. In this work, as the taxonomic status was expected to be similar for the different Russell lupin genotypes, attention was directed to examine continuous variation based on agronomic attributes.

Comparisons of the different statistical techniques was beyond the scope of this thesis. From the literature it appears that, PCA, factor analysis, canonical correlation analysis, and CVA are all related, from the point of dimension, or data reduction. However, PCA and factor analysis are mostly used to show the interrelationships of quantitative variables. Canonical correlation analysis is used to assess the relationships between two sets of variables, where each set can contain several variables. Canonical variate analysis on the other hand, optimally separate groups if individuals belong to distinct groups, such as the genotypes in the present study. Thomson (1974) successfully studied components of winter hardiness in a collection of ryegrass varieties in Britain using canonical variate analysis. The present study demonstrated that canonical variate analysis is a useful tool for characterization of the pattern of variability of accessions in a

germplasm collection. Germplasm description and characterization studies are indispensable if collected genetic resources are to be effectively utilised by plant breedres and agronomists.

5.4 SUMMARY AND FUTURE WORK

The overall objective of this research project was to improve the information base on Russell lupins, and to provide data useful to others for future breeding and agronomic work on this plant.

Extrapolation of the results from the seedling emergence studies in the glasshouse should provide guidelines for optimum field establishment of Russell lupin.

Further, the field experiment facilitated morphological description and characterization of 18 Russell lupin accessions from six different countries. The number of accessions has now been reduced to four distinct groups. The variables, that had the most discriminatory effect in showing the relationships of the accessions, appear to be both agronomically and biologically useful for future breeding and agronomic programmes on the Russell lupin in New Zealand. Results that have been developed from this research are:-

(1) Hand harvested Russell lupin seed should be scarified in concentrated sulphuric acid (36N), for 30 to 45 minutes immersion. This should produce 75% or more emerged normal seedlings.

(2) Scarified seed should be sown at 1 to 2 cm. However, stock trampling of sown seed may have to be considered in hilly areas where drilling is impractical, although establishment would probably be reduced.

(3) In controlled environment cabinets at constant temperature, the optimum temperature for maximum seedling emergence appears to be in the range of 10 to 20 ^{o}C. Providing soil moisture is adequate, this suggests that best field emergence of Russell lupin would result from sowings in early autumn (March/April) or mid spring (September/October).

(4) Russell lupins are highly variable in their morphology, growth, performance, and floral characteristics.

(5) As the number of distinct genotypes in the germplasm collection has now been reduced to four, further agronomic and breeding work can be based on a single representative from each group.

(6) Two accessions, which are already adapted to the New Zealand environment, with higher yield potential than Connie and the mean of the 18 accessions have been identified. These were ON and HN both New Zealand collections. Their different flowering response, perhaps to cool conditions, should provide a good opportunity for future breeding and agronomic work on the Russell lupin in New Zealand.

Further work is necessary to further improve the information base on the Russell lupin as an alternative forage legume species for the hill and high country of New Zealand. These are listed below.

(1) The remarkable growth and performance of almost all accessions in a dry to very dry year suggest that the Russell lupin is tolerant of seasonal drought. This may have been due to a deep root system. A physiological study to examine the plant's reaction to water stress would provide insights into why Russell lupin is drought tolerant. Further, study on the Russell lupin's root systems would provide additional information on the plant's water use.

(2) Special-purpose characterization, edaphic adaptation, and further evaluation studies need to be undertaken on hill and high country environments using only representative accessions from each group.

(3) To define the minimum temperature requirement for seed germination, seedling emergence and plant survival, a study should be undertaken to assess the effects of a range of low temperatures on Russell lupin.

(4) To confirm the establishment guidelines developed from the glasshouse studies, a study on the field establishment of Russell lupin is required.

(5) Determination of the effect of temperature and photoperiod on flowering in Russell lupin is required.

(6) As one of the primary roles of Russell lupin in the hill and high country will be to increase nitrogen in the soil for grasses, research on the optimisation of nitrogen fixation is required.

(7) Determination of the distribution of plant components, dry matter yield and nutritional quality is also important. This should include alkaloid levels, digestibility, crude protein and fibre.

(8) In the long term, studies also need to be taken on animal performance on the crop. determination of individual plants over the different growing stages.

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APPENDIX 1

Table 1. The effect of method of scarification on the germination of Russell lupin seeds after 10 days in a seed germinator set at a constant temperature of 20^0C.

Acid immersion (minutes)	Germination (%)
0	34.5
15	67.5
30	86.5
45	79.5
60	84.5
90	88.0
120	88.0
Chipping	98.0
Significance	* * *
$LSD_{(0.05)}$	9.10

APPENDIX 1

Table 2. The effect of method of scarification on percentage emergence of total seedlings of Russell lupin in a glasshouse. Data recorded at different intervals of time. Results are mean emergence percentages of three replicates.

Immersion in acid (minutes)	DAYS AFTER SOWING 6	7	8	9	10	15
0	1.33	6.67	10.33	15.00	16.67	40.00
15	5.33	23.33	40.00	55.67	63.67	72.67
30	5.67	35.00	56.00	73.67	79.00	85.67
45	5.33	35.00	57.67	76.00	83.67	89.00
60	6.33	36.00	59.00	71.00	76.00	83.00
75	2.67	31.00	49.00	69.33	75.33	83.33
90	5.33	29.33	48.67	64.33	71.00	81.33
105	3.00	27.33	43.33	63.33	72.33	79.67
120	2.00	16.33	34.67	55.33	64.67	73.00
135	4.00	17.33	35.33	54.67	62.00	70.00
150	2.00	12.67	27.00	44.33	55.33	69.67
165	2.67	15.33	29.67	46.00	54.00	64.33
180	3.33	11.33	27.00	48.00	55.00	64.67
Chipping	9.67	48.00	68.67	85.00	90.00	92.00
Significance	ns	***	***	***	***	***
$LSD_{(0.05)}$	4.89	10.50	11.80	10.50	9.22	8.68

APPENDIX 1

Table 3. The effect of method of scarification on percentage emergence of abnormal seedlings of Russell lupin seeds in a glasshouse. Data recorded at different intervals of time. Results are mean emergence percentages of three replicates.

Immersion in acid (minutes)	DAYS AFTER SOWING 6	7	8	9	10	15
0	0.00	0.00	0.00	0.00	0.33	0.33
15	0.00	0.33	1.33	3.33	4.33	7.00
30	0.00	0.67	1.67	3.67	4.67	6.33
45	0.33	0.67	1.67	4.67	6.33	9.33
60	0.00	0.67	2.67	5.67	7.00	12.33
75	0.33	2.00	4.67	10.33	13.33	18.33
90	0.33	2.67	5.00	9.33	11.33	15.67
105	0.00	1.67	4.67	13.33	18.67	23.67
120	0.33	2.00	3.67	10.67	15.67	21.67
135	0.00	1.67	3.00	9.33	11.33	15.33
150	0.67	2.00	4.33	11.33	17.67	27.67
165	0.00	3.00	6.67	13.33	17.67	26.33
180	0.33	2.00	7.67	18.67	23.00	32.33
Chipping	2.33	18.00	26.00	33.00	35.00	36.00
Significance	ns	***	***	***	***	***
$LSD_{(0.05)}$	1.25	4.40	5.90	6.18	5.87	7.52

APPENDIX 1

Table 4. The effect of method of scarification on percentage emergence of normal seedlings of Russell lupin in a glasshouse. Data recorded at different intervals of time. Results are mean emergence percentages of three replicates.

Immersion in acid (minutes)	DAYS AFTER SOWING 6	7	8	9	10	15
0	1.33	6.67	10.33	15.00	16.33	39.67
15	5.33	23.00	38.67	52.33	59.33	65.67
30	5.67	34.33	54.33	70.00	74.33	79.33
45	5.00	34.33	56.00	71.33	77.33	79.67
60	6.33	35.33	56.33	65.33	69.00	71.00
75	2.33	29.00	44.33	59.00	62.00	65.00
90	5.00	29.67	43.67	55.00	59.67	65.67
105	3.00	25.67	38.67	50.00	53.67	56.00
120	1.67	14.33	31.00	44.67	49.00	51.33
135	4.00	15.67	32.33	45.33	50.67	54.67
150	1.33	10.67	22.67	33.00	37.67	42.00
165	2.67	12.33	23.00	32.67	36.33	38.00
180	3.00	9.33	19.33	29.33	32.00	32.33
Chipping	7.33	30.00	42.67	52.00	55.00	56.00
Significance		***	***	***	***	***
$LSD_{(0.05)}$	4.39	9.05	10.38	9.31	10.35	11.29

Appendix 2

Table 1 Raw data from sample plants of Russell lupin genotypes grown in Canterbury from December 1987 to November 1988. Measurements taken in Autumn 1988.

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
1	C	1	41	21	19	11	2.2	9	50.9	0
1	C	2	43	23	19	11	2.4	14	21.8	0
1	C	3	44	55	15.3	8	2.0	10	112.8	1
1	C	4	35	22	20	9.5	2.3	13	15.2	0
1	C	5	48	26	22.5	12.4	2.6	12	76.3	0
2	B	1	38	40.5	14	6.8	2.5	11	21.7	1
2	B	2	37	37	11	5	1.3	7	28.1	1
2	B	3	36	21	19.3	8.5	2.8	8	32.8	0
2	B	4	52	44	17.2	9.7	2.0	9	7.8	1
2	B	5	50	53	12.8	6.6	2.0	6	31.9	1
4	D	1	43	25	20	9.5	2.1	11	22.9	0
4	D	2	39	55	15.3	8.5	2.6	10	24.4	1
4	D	3	45	40	12.8	8	1.8	11	25.1	1
4	D	4	41	27	19.4	10	2.3	12	32.5	0
4	D	5	47	27	24	13	2.5	13	25	0
5	E	1	48	27	21	10.9	3	12	53.6	0
5	E	2	45	29	22.4	11	2.7	11	69	0
5	E	3	49	27	24	12	3	12	65.6	0
5	E	4	57	28	25	13.3	3.2	13	55.7	1
5	E	5	42	27	19.1	11.4	2.6	10	24.1	0
6	F	1	50	27	21.5	11	2.3	12	73.6	0
6	F	2	54	65	18	9.5	3.2	10	98	1
6	F	3	48	26	19.1	11	3	13	31.5	0
6	F	4	50	43	19.2	9.5	3	11	80.2	1
6	F	5	41	25	24.3	13.5	4.4	13	55.8	0
7	C	1	39	57	15.7	8.4	2.8	9	51.6	1
7	C	2	50	49	15.1	7.7	2.4	8	93.3	1

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
7	C	3	56	50	15.3	8	2.1	11	81.5	1
7	C	4	56	47	15.4	8.3	2.2	7	89.6	1
7	C	5	49	23	17.7	9.6	2.8	12	121.6	0
8	G	1	49	44	25.7	12.7	3.5	12	50.6	1
8	G	2	47	28	19	9.5	2.8	11	23.4	0
8	G	3	58	30	21.1	10.3	2.3	12	43.2	0
8	G	4	57	30	28	14	3.2	13	74	0
8	G	5	48	29	24	10.8	2.5	12	33.1	0
9	H	1	46	59	18.2	10	2.1	10	43	1
9	H	2	47	64	17.5	10	3.1	12	113.0	1
9	H	3	45	31	20.1	10.3	2.8	10	62.0	1
9	H	4	42	56	16.2	8	2.0	12	63	1
9	H	5	46	59	21	11	3.2	12	153.8	1
10	I	1	47	26	23	12	3	13	23.3	0
10	I	2	36	27	22.2	12.1	3	12	32.7	1
10	I	3	39	23	19.5	11	2.4	11	19.5	0
10	I	4	38	27	19.2	10	2.3	13	5.1	0
10	I	5	39	31	24.3	12	2.8	13	16.6	0
11	J	1	42	22	18.7	10	2.1	14	24.5	0
11	J	2	32	52	15.3	7.8	2	11	35.9	1
11	J	3	60	26	25	13.2	2.7	14	93	0
11	J	4	45	26	24.5	12.5	2.3	13	28.4	0
11	J	5	40	20	21.3	11	2.2	16	33	0
12	K	1	40	22	22.5	11.7	3.1	12	34.3	0
12	K	2	41	51	19.1	11	2.2	14	21.8	1
12	K	3	45	27	23.3	12	2.6	12	34.3	0
12	K	4	44	26	27	14.1	2.8	13	38.0	0
12	K	5	46	34	21.8	12.1	3.1	12	48.5	0
13	C	1	48	56	18.5	9.5	2	11	33.7	1
13	C	2	47	43	11.1	6.5	1.8	11	30.3	1
13	C	3	44	28	17.7	9.1	2.4	10	11.5	1
13	C	4	45	41	17.6	9	2.4	14	80	1
13	C	5	47	39	17.4	8.5	2.4	12	52.8	1
14	L	1	40	58	15.4	8.6	2.5	9	31.4	1
14	L	2	47	30	25.3	12.5	3.2	11	38.5	0
14	L	3	36	22	20.0	9.1	2	12	4.9	0

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
14	L	4	38	26	18	9	2.2	8	29.3	0
14	L	5	39	28	22.8	12.4	2.1	14	47.8	0
15	M	1	51	24	25.5	14	2.6	10	76.4	0
15	M	2	52	23	24.8	13	3.4	13	58.5	1
15	M	3	41	23	23	11.5	3.1	11	29.5	1
15	M	4	37	19	18	9.4	1.9	13	37.8	0
15	M	5	43	27	20	9.4	2.2	11	38.4	1
16	N	1	41	27	22.8	11	3	13	69.7	0
16	N	2	58	54	22.7	12.3	2.3	12	42.6	1
16	N	3	60	22	24	13	3	13	49.8	0
16	N	4	50	33	19.2	10.4	2.1	12	59.7	1
16	N	5	48	51	21.5	12	3.8	10	57.6	1
17	O	1	53	29	21.5	10.6	2.9	15	63	1
17	O	2	51	30	23	12	2.7	12	58.7	0
17	O	3	61	30	22.2	12.3	2.7	13	38.5	0
17	O	4	55	26	18	10	3	12	74.3	0
17	O	5	61	61	17.8	10	3	14	23.1	1
18	P	1	51	34	23.5	12	2.5	9	43.4	1
18	P	2	47	30	21	11	2.8	12	46.7	0
18	P	3	48	53	14.2	8	2.2	9	52.3	1
18	P	4	61	51	25.7	14	2.2	13	58.8	1
18	P	5	45	61	16.1	8.2	2.3	10	32.3	1
19	C	1	50	37	15.8	8.4	2.2	11	76.3	1
19	C	2	41	46	13.6	7.6	1.6	9	17.5	1
19	C	3	55	25	17	9	2.5	10	90.4	1
19	C	4	50	40	13	6	1.6	12	82.5	1
19	C	5	51	54.5	12.5	6.5	1.6	12	32.5	1
20	Q	1	44	23	23.8	11	2.9	14	40.5	0
20	Q	2	56	27	19	9	2.2	12	46.5	0
20	Q	3	52	25	21.3	11.6	2.3	12	21.5	0
20	Q	4	26	16	13	7.5	2	11	63.0	0
21	R	1	43	25	23.3	12.1	3.2	10	42	1
21	R	2	47	23	20.5	11.1	2.6	12	56.2	1
21	R	3	44	24	21.3	11	3	12	61.7	1
21	R	4	47	46	11.7	6	1.5	9	141.8	1
21	R	5	44	23	18.1	8.8	2	11	46.0	0

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
22	S	1	38	19	22.5	11.2	2.6	13	16.9	1
22	S	2	43	22	19.5	11.1	2.7	11	17.4	0
22	S	3	34	22	20.2	11	2.5	11	15.2	0
22	S	4	49	22	18.3	10	2.8	13	19.1	0
22	S	5	45	28	15.7	7.3	2.5	13	51	0
23	C	1	47	19	21.4	11	3.1	13	35.7	1
23	C	2	39	50	18.1	9	2.3	11	64	1
23	C	3	35	43	17.1	8.4	2.6	9	13.3	1
23	C	4	43	40	14.5	8.6	2.4	10	27	1
23	C	5	40	31	19.1	10.2	1.6	12	16.8	1
24	L	1	41	26	19.9	11.5	3.4	14	31.1	0
24	L	2	43	23	22	12.1	2.8	14	37.5	0
24	L	3	42	25	21	12	2	12	25.5	0
24	L	4	30	24	16.3	8.5	1.6	14	10.6	0
24	L	5	39	27	21.4	12.5	2.6	10	31.0	0
25	G	1	44	26	23.3	12.2	3	13	32.4	0
25	G	2	47	26	20.6	10.1	2	12	20.9	0
25	G	3	37	24	21.1	10	2.7	12	46.8	0
25	G	4	35	25	20.9	12.4	2.1	13	11.6	0
25	G	5	45	25	21.3	11.6	2.2	14	16.4	0
26	F	1	43	20	23.2	10.3	3.7	12	41.3	1
26	F	2	44	24	16.6	8.5	2.3	13	65.8	0
26	F	3	35	18	19.5	8.8	3.0	13	24.5	0
26	F	4	36	22	15.3	8	2.2	9	24.6	0
26	F	5	35	17	15	7.6	1.8	11	21.0	0
27	E	1	42	27	23	12.7	1.8	12	13.6	0
27	E	2	46	26	21.5	11.1	3.1	13	59.2	0
27	E	3	53	31	22.1	13.1	2.2	11	29.5	0
27	E	4	41	21	21	11.7	2.3	10	54.4	0
27	E	5	36	21	22.3	10.9	1.6	13	5.6	0
28	B	1	42	24	17.6	9.5	2.1	10	47.5	1
28	B	2	47	44	15	7.8	2.3	11	60.8	1
28	B	3	32	20	14.4	6.6	1.7	11	6.6	0
28	B	4	40	20	19	9.4	2.3	12	39.0	0
28	B	5	35	39	20.8	11.1	2.9	9	42.6	1
29	C	1	47	73	18	10	2.6	13	99	1

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
29	C	2	31	21	27.4	13.5	3.5	12	15.8	0
29	C	3	41	22	26.3	12	4.3	13	94.8	0
29	C	4	55	24	21.1	10	3.6	11	80.3	0
29	C	5	47	21	16.2	9.4	3	13	31.0	0
30	O	1	40	28	15.8	8.3	2.3	10	31.6	0
30	O	2	47	20	17.1	8.6	2.7	11	53.0	0
30	O	3	62	21	21.7	10.8	2.3	13	50.3	0
30	O	4	39	20	16.2	8.5	2.3	12	19.7	0
30	O	5	42	23	24	12.5	2.6	13	41.1	0
31	D	1	53	54	20	10.8	2.6	10	56	1
31	D	2	48	28	21.1	11.5	2.5	11	44.8	0
31	D	3	46	23	23.5	12	2.4	13	25.6	0
31	D	4	32	16	18.4	9.6	2.5	11	13.8	0
31	D	5	42	49	17.5	11	2	12	22.7	1
32	M	1	41	19	20.5	10	2.6	10	54.3	0
32	M	2	42	22	21	11	2.5	12	34.5	0
32	M	3	40	20	18.2	9.4	2.3	13	23.7	0
32	M	4	49	25	19.4	10.7	3.2	12	72.4	0
32	M	5	45	23	19.8	10.1	2.6	12	37.3	0
33	N	1	49	22	27	14	3.3	10	81.8	0
33	N	2	40	44	23	12	3.3	11	29.8	1
33	N	3	42	24	21.8	11.3	2.3	12	35.9	0
33	N	4	41	22	20.7	11	3	14	35.3	0
33	N	5	39	39	19	10	4.4	13	47.7	1
34	I	1	44	18	21	10.7	2	12	17.4	0
34	I	2	34	16	15	8.5	1.7	11	4	0
34	I	3	42	26	22	12.8	2.6	12	23.3	0
34	I	4	37	24	15.7	9	1.8	13	4.9	0
34	I	5	50	25	26	13.5	2.3	12	6	0
35	C	1	36	19	17.5	9.2	2.1	13	29.6	0
35	C	2	42	25	16	8.3	3	10	53.3	0
35	C	3	40	24	20	10.1	2	12	51.8	0
35	C	4	46	48	20.1	10.5	2.8	12	67.9	1
35	C	5	44	21	20.1	9.6	2.1	11	96.8	0
36	P	1	40	25	22	11.8	2.7	11	49.4	0
36	P	2	41	27	17.3	9.6	2.4	12	26	0

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
36	P	3	30	24	16.8	9.1	2.0	11	21.2	0
36	P	4	40	24	18	9.4	2.6	12	33.8	0
36	P	5	45	37	25.8	12.2	2.8	12	39.6	1
37	J	1	41	34	20	11.6	2.8	12	42.5	1
37	J	2	42	19	19.5	11	2.3	13	14.0	0
37	J	3	37	22	18.3	9.7	2.4	10	23.7	0
37	J	4	41	28	21.6	11.6	2.6	14	44.5	0
37	J	5	43	22	21.8	11	2.9	14	24.8	0
39	H	1	45	47	17	8.6	3.3	12	33.2	1
39	H	2	35	27	22.3	11.1	2.5	14	61.5	0
39	H	3	27	36	14.3	7.4	2.2	10	27.2	1
39	H	4	48	27	22	11.2	2.3	14	56.3	0
39	H	5	44	65	17.1	9.5	2.4	10	53.4	1
40	K	1	39	22	22.8	11.7	2.6	14	19.3	0
40	K	2	41	27	25	12.2	2.7	13	44.7	0
40	K	3	49	21	19.5	10.6	2.5	12	42.1	0
40	K	4	44	27	24.3	13	3.1	14	55	0
40	K	5	37	21	18.4	10.4	2.3	11	33.4	0
41	C	1	43	45	14	8.3	2.4	11	58	1
41	C	2	50	36	17	8.5	2.2	11	52.1	1
41	C	3	41	21	17.8	9.4	3.7	12	67	0
41	C	4	48	44	19	10	2.5	12	63.8	1
41	C	5	37	17	17.3	9.4	2.3	10	28.1	0
42	S	1	58	34	19	10.2	2.4	13	73.1	1
42	S	2	55	25	22	13	2	13	25.6	0
42	S	3	45	20	18.5	10	2	13	36.7	0
42	S	4	40	25	19.7	11.5	2.2	11	29.5	0
42	S	5	49	56	21.1	12	2.7	12	57.8	1
43	Q	1	28	18	17	9.1	2.3	12	13.3	0
43	Q	2	40	21	20.6	10.5	3	12	55.3	0
43	Q	3	30	16	13.6	7	1.3	11	9.0	0
43	Q	4	43	24	21.1	10.2	2.5	12	32.1	0
44	R	1	30	21	14.8	7.0	2	11	22.4	0
44	R	2	43	55	18.6	9.1	3	12	33.3	1
44	R	3	31	23	12.7	6.7	1.7	12	17.1	1
44	R	4	27	16	17.2	8.5	1.8	12	6.9	0

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
44	R	5	25	18	12.7	6.1	1.6	11	19.1	0
45	C	1	44	51	16.3	8.2	1.8	10	47.7	1
45	C	2	32	14	14.8	7.4	1.6	12	11.8	0
45	C	3	29	47	13.3	7.6	2	10	18.8	1
45	C	4	33	46	12.1	6.8	1.9	10	17.4	1
45	C	5	36	18	15.5	9	2.3	10	23.5	0
46	J	1	55	24	17	8.8	1.7	11	47	0
46	J	2	42	26	22.8	11.5	2.4	14	23.6	0
46	J	3	38	22	20.2	11	2.4	13	21.2	0
46	J	4	32	19	17.4	9	2	17	14.4	0
46	J	5	33	19	17	9.7	2.3	13	13.0	0
47	P	1	41	21	22.7	11	2.8	12	16.6	0
47	P	2	52	59	18.6	10	2.7	10	58.5	1
47	P	3	30	20	17.4	8.8	2.3	12	25.2	0
47	P	4	39	25	21.2	10.7	2.2	13	17.3	0
47	P	5	35	23	21.5	10.5	3.3	11	28.6	0
48	E	1	41	21	19	10.2	2	12	7.6	0
48	E	2	45	22	22.2	10.9	2.2	12	25.9	0
48	E	3	40	23.5	20	10.5	2.9	12	26.2	0
48	E	4	49	27	16.5	8.5	2.8	10	47.3	0
48	E	5	38	43	16	8.3	2.4	11	17.8	1
50	G	1	52	26	21	11.1	2.3	12	36.2	0
50	G	2	41	25	23.5	11.4	2.8	11	31.1	0
50	G	3	42	23	22.5	13	3.6	13	24.2	0
50	G	4	49	23	20.2	11.5	2.8	11	16.8	0
50	G	5	38	25	19.5	10.1	2.2	12	15.1	0
51	C	1	40	38	20	9.5	2	11	28.4	1
51	C	2	45	38	15.6	8.2	2.4	13	34.2	1
51	C	3	49	37	16.5	9.1	2.4	11	49	1
51	C	4	20	20	15.2	8.2	2	12	17.1	0
51	C	5	47	22	15.5	8.3	2	11	24.1	0
52	K	1	47	25	23.3	13	2.7	11	31.4	0
52	K	2	44	24	25.3	12.2	4.0	11	30.7	1
52	K	3	49	25	22.2	11.7	2.5	12	58	1
52	K	4	50	55	19.8	10.5	3	10	76.1	1
52	K	5	46	31	17.2	9	3	14	33.2	1

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
53	H	1	43	49	18.7	10.5	2.2	10	49.9	1
53	H	2	42	50	14.5	8.5	2.3	10	95.1	1
53	H	3	44	67	19.2	9.3	2.8	10	84.9	1
53	H	4	45	53	19	10	2.6	14	83	1
53	H	5	44	26	20.1	11.3	2.5	12	75.2	0
54	D	1	45	28	24.5	14.5	2.6	10	43.6	0
54	D	2	53	23	20.2	11.5	3	12	16.5	0
54	D	3	44	30	20.7	10.7	2.7	14	53.7	0
54	D	4	55	64	19.3	11	3	12	56	1
54	D	5	48	24	20.1	10.6	2	12	34.7	1
55	B	1	40	36	15	7.5	1.8	11	54.3	1
55	B	2	40	37	14.1	7	2	11	39	1
55	B	3	40	35	24	12	2.5	12	35.9	1
55	B	4	36	19	18	10.1	2.1	12	19	1
55	B	5	40	50	22	11	2.6	10	35.6	0
56	O	1	57	28	19.8	10.2	3.7	14	36.1	0
56	O	2	52	31	18.5	11.2	2.2	15	5	0
56	O	3	50	29	26	12.8	2.8	12	37.9	0
56	O	4	38	24	20.5	10.2	3	12	43.2	1
56	O	5	38	23	21.7	10.5	3.0	12	22.3	0
57	C	1	39	45	16	7.5	2	10	52.5	1
57	C	2	54	37	23	12.7	3.7	10	39.2	1
57	C	3	47	52	15.2	7.8	2.7	11	32.2	1
57	C	4	55	26	22.5	11.8	3.3	12	79.6	0
57	C	5	46	44	17	8.5	2.7	11	24.7	1
58	F	1	41	22	19.3	9	2	11	31.7	0
58	F	2	41	21	19	9	2.5	13	52	0
58	F	3	50	20	18.3	11.1	2.5	11	34.9	0
58	F	4	36	37	17	9	2.6	12	31	1
58	F	5	32	27	15.9	8	2.3	12	26.4	0
59	I	1	49	57	18.2	11.0	2.7	13	30.7	1
59	I	2	48	24	16	9	1.5	11	11.3	0
59	I	3	34	22	20.2	9.5	2.2	11	11.9	0
59	I	4	44	41	21.5	11	3.3	13	23.4	1
59	I	5	44	20	22	12	2.4	12	23.4	0
60	M	1	40	21	20.8	11	2.5	10	34.6	0

Plot No.	Accession Code	Sample NO.	Plant Spread (cm)	Plant Height (cm)	Leaf Diameter (cm)	Leaflet Length (cm)	Leaflet Width (cm)	Leaflet Number	Dry Matter (g)	Autumn Flowering (score)
60	M	2	42	47	17	9	2.5	10	30	1
60	M	3	52	25	21	11	3	12	77.8	0
60	M	4	47	26	22.5	11.3	2.5	12	37.9	0
60	M	5	46	26	22.5	13	2.5	13	35.1	0
61	L	1	47	21	23	12.5	2.5	11	32	0
61	L	2	38	25	22.5	13.7	3	10	33.5	0
61	L	3	50	23	23.4	12	2.5	13	48.7	0
61	L	4	41	23	20	10.7	2.3	11	24.8	0
61	L	5	42	21	22.4	11	2.3	12	17.7	0
62	N	1	54	24	22	10.3	3	12	27.3	1
62	N	2	40	23	20	10.7	3.5	16	25.4	0
62	N	3	42	27	19.7	9	3.4	14	73.9	1
62	N	4	53	24	17.0	9	2	11	70.8	1
62	N	5	47	27	21.7	11	2.4	12	21.9	0
63	C	1	46	48	15.5	8.5	2.6	11	31.9	1
63	C	2	45	53	17	8	2.2	11	66.7	1
63	C	3	52	35	16.7	8.6	2.4	13	56	1
63	C	4	53	24	22	11.5	2.5	14	34	0
63	C	5	40	41	15.7	7.8	2	10	19.6	1
64	R	1	34	18	16	9.2	1.8	11	43.5	0
64	R	2	42	46	14	9.0	1.6	9	78.9	1
64	R	3	32	22	17.5	8.3	1.8	12	31.3	1
64	R	4	43	23	19.3	9.5	2.3	11	22.6	0
64	R	5	43	27	18.2	9	2.6	13	38.3	0
65	S	1	45	22	23.2	12	3.1	10	27.3	0
65	S	2	40	61	20.5	10.0	3	12	12.7	1
65	S	3	32	21	19.2	10.5	2.2	14	20.5	0
65	S	4	42	21	18.6	10.0	2.5	12	15.5	1
65	S	5	38	22	19.7	10	2.1	12	17.9	0
66	Q	1	50	21	22.7	11.5	2.5	11	31.4	0
66	Q	2	36	19	22	13.2	1.7	14	14.9	0
66	Q	3	42	20	18.1	10	2.3	12	28.0	0
66	Q	4	34	22	19.2	11	2.5	13	25.3	1

Appendix 2

Table 2 Raw data from sample plants of Russell lupin genotypes grown in Canterbury from December 1987 to November 1988. Measurements taken during spring 1988.

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
1	1	1	*	*	*	*	*	*	*	*	*	*	*	*
1	1	2	*	*	*	*	*	*	*	*	*	*	*	*
1	1	3	80	83	38	1.4	11	12.5	7.0	1.5	7	3	320.0	69
1	1	4	75	66	31	1.5	12	11.0	6.0	1.7	8	3	134.1	300
1	1	5	81	96	38	1.4	14	12.6	6.5	1.4	3	3	501.1	292
2	1	1	60	75	37	1.5	14	11.0	6.0	1.7	1	3	134.9	47
2	1	2	57	72	29	1.2	13	12.4	6.0	1.0	1	3	109.2	54
2	1	3	75	79	29	1.4	12	9.0	5.0	1.5	1	3	188.8	307
2	1	4	70	77	33	1.0	13	15.0	8.0	1.3	1	3	211.4	54
2	1	5	70	50	20	1.2	12	11.0	6.0	1.5	3	3	124.4	54
4	1	1	55	85	46	1.4	13	12.5	7.5	1.5	9	3	123.0	299
4	1	2	76	83	42	1.1	12	13.0	8.5	1.7	9	3	202.1	56
4	1	3	64	75	34	1.2	10	16.0	8.5	2.0	9	3	115.0	63
4	1	4	80	85	38	1.3	12	12.5	6.7	1.4	9	3	189.4	300
4	1	5	88	76	32	1.1	14	12.0	6.5	1.4	1	3	172.4	319
5	1	1	75	98	34	1.3	14	13.2	7.0	1.3	9	3	396.0	299
5	1	2	70	100	35	1.2	13	12.0	6.0	1.7	9	3	277.1	298
5	1	3	80	100	36	1.1	13	19.2	8.5	2.0	9	3	321.0	294
5	1	4	100	120	45	1.3	15	16.0	8.5	2.0	9	3	308.0	81
5	1	5	95	105	47	1.9	12	17.5	10.5	2.0	9	3	304.9	299
6	1	1	70	82	30	1.1	12	14.2	8.0	2.0	3	3	255.5	314
6	1	2	100	100	52	1.8	13	13.0	7.2	1.9	5	3	255.1	64
6	1	3	96	95	27	1.4	16	12.0	7.0	2.0	2	3	254.5	312
6	1	4	85	101	46	1.3	13	16.2	8.5	2.6	5	3	266.5	56
6	1	5	80	98	40	2.1	10	13.0	7.5	2.0	5	3	233.2	300
7	1	1	70	88	33	1.2	15	12.0	6.5	1.6	1	3	218.1	56
7	1	2	90	90	36	1.3	14	12.5	7.0	1.8	1	3	300.8	54

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
7	1	3	78	96	30	1.3	13	16.0	9.0	2.1	3	3	297.0	53
7	1	4	90	92	35	1.4	12	19.5	10.5	2.2	7	3	372.5	52
7	1	5	93	85	37	1.0	12	11.0	6.0	1.4	5	3	236.0	301
8	1	1	73	97	37	1.4	13	15.5	8.5	2.0	3	3	283.3	76
8	1	2	74	85	36	1.0	12	10.5	6.5	1.5	9	3	179.8	278
8	1	3	90	94	39	0.9	11	14.5	8.5	2.0	9	3	312.4	285
8	1	4	100	100	43	1.0	14	12.5	6.5	1.3	9	3	436.4	292
8	1	5	100	110	50	1.2	12	13.0	8.0	1.5	9	3	327.4	285
9	1	1	70	97	41	1.4	14	14.5	7.5	1.6	9	3	243.2	56
9	1	2	85	100	27	1.5	14	11.0	5.5	1.5	9	3	255.2	67
9	1	3	100	115	55	1.7	12	17.5	9.5	2.2	9	3	392.8	66
9	1	4	90	110	41	1.5	12	16.0	8.5	2.2	9	3	407.7	69
9	1	5	85	100	33	1.5	12	13.5	7.6	1.7	9	3	414.8	69
10	1	1	80	80	33	1.2	13	13.0	7.0	1.4	9	3	224.4	298
10	1	2	90	91	39	1.1	13	13.5	6.5	1.3	9	1	145.3	82
10	1	3	51	78	35	1.3	10	*	*	*	9	2	48.2	292
10	1	4	60	73	31	0.8	14	10.7	6.1	1.2	9	2	51.4	301
10	1	5	92	96	39	1.1	14	16.0	8.3	2.0	9	2	155.5	301
11	1	1	80	88	36	1.0	15	14.0	7.7	1.7	9	3	358.0	288
11	1	2	84	108	49	1.6	12	18.2	10.0	2.0	9	3	189.9	68
11	1	3	100	107	37	1.2	13	15.0	8.0	2.2	9	3	594.4	279
11	1	4	98	97	38	1.2	10	10.6	6.0	1.7	9	3	341.0	298
11	1	5	85	91	39	0.9	11	16.0	10.3	1.4	9	3	204.1	293
12	1	1	74	82	34	1.4	11	12.4	8.1	1.6	9	3	161.1	294
12	1	2	78	73	30	1.3	14	13.5	7.5	1.5	9	3	199.0	72
12	1	3	84	79	29	1.1	14	10.0	5.4	1.1	9	2	171.9	317
12	1	4	82	88	30	1.3	10	12.0	6.7	1.3	9	3	242.7	288
12	1	5	78	77	33	1.0	11	11.6	6.4	1.5	9	3	220.5	293
13	1	1	90	94	38	1.3	15	12.0	7.3	2.1	1	3	357.0	52
13	1	2	67	78	33	1.5	15	9.0	5.0	1.4	1	3	159.8	56
13	1	3	78	95	39	0.9	15	12.2	6.6	1.4	7	3	226.3	64
13	1	4	71	78	33	1.5	13	10.1	6.0	1.6	3	3	193.2	69
13	1	5	82	71	27	0.9	11	13.5	7.3	1.8	1	3	293.7	73
14	1	1	72	83	30	1.2	11	12.2	7.1	1.7	1	3	187.2	54
14	1	2	75	99	44	1.8	13	16.0	8.5	1.7	1	3	303.9	292
14	1	3	48	82	30	0.9	11	10.2	5.3	1.0	1	3	64.8	315

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
14	1	4	60	100	37	1.2	15	11.0	5.7	1.2	9	3	219.7	300
14	1	5	90	93	36	1.2	13	16.2	9.3	1.3	9	3	312.0	288
15	1	1	88	93	33	1.1	12	11.8	8.0	1.5	1	3	359.0	302
15	1	2	100	99	47	1.6	13	13.0	7.6	1.9	1	3	319.9	88
15	1	3	75	91	44	1.1	12	12.3	7.1	1.6	7	3	145.4	86
15	1	4	75	79	33	1.0	11	13.6	8.1	2.5	3	3	207.0	300
15	1	5	81	95	42	1.2	11	17.4	9.3	2.0	9	3	180.1	87
16	1	1	99	92	35	1.0	12	13.1	7.3	1.7	9	3	494.3	300
16	1	2	84	106	38	0.9	14	14.3	8.1	1.5	1	3	511.1	73
16	1	3	100	90	32	1.5	12	17.0	10.2	2.5	9	3	377.0	300
16	1	4	89	116	39	1.5	13	14.4	8.0	2.0	1	3	316.8	83
16	1	5	100	116	58	1.0	13	16.7	10.0	2.0	9	3	400.3	76
17	1	1	80	97	40	1.3	14	14.1	7.6	1.6	9	3	338.0	94
17	1	2	81	118	38	1.4	15	12.3	7.0	2.0	9	3	324.5	294
17	1	3	92	97	36	1.4	14	16.7	8.8	2.2	9	3	253.2	300
17	1	4	81	111	40	1.8	11	11.6	6.7	1.5	9	3	227.1	279
17	1	5	100	97	30	1.1	13	14.1	7.5	2.1	9	3	673.7	62
18	1	1	78	91	38	1.1	12	8.7	5.5	1.0	9	3	265.7	76
18	1	2	84	98	32	1.1	14	13.5	7.3	1.6	9	3	339.7	292
18	1	3	73	100	44	0.9	11	13.0	7.6	2.1	3	3	184.4	53
18	1	4	69	94	44	1.3	15	11.1	6.6	1.7	3	3	238.5	73
18	1	5	80	123	47	1.2	11	16.1	9.6	2.1	2	3	232.3	69
19	1	1	100	95	34	0.9	12	16.2	8.5	2.7	9	3	532.9	63
19	1	2	54	96	46	1.5	9	15.1	8.7	2.0	2	3	75.2	52
19	1	3	97	91	38	1.6	11	10.1	5.0	1.3	9	3	534.1	102
19	1	4	100	73	33	1.4	14	13.7	7.2	1.5	10	3	395.1	58
19	1	5	84	73	26	1.1	14	11.0	5.6	1.0	1	3	134.9	52
20	1	1	74	92	44	0.9	14	15.0	7.0	1.4	9	3	234.1	315
20	1	2	95	91	37	1.3	11	15.3	8.5	2.0	9	3	365.6	300
20	1	3	87	100	38	1.1	11	14.3	7.4	1.6	9	3	333.2	276
20	1	4	75	62	60	0.7	12	9.1	5.2	1.1	9	3	78.6	300
21	1	1	85	41	*	*	16	17.0	9.0	2.8	*	*	123.5	102
21	1	2	86	89	42	1.1	12	15.5	7.6	1.8	1	3	322.0	82
21	1	3	86	79	44	1.0	11	15.1	9.2	2.2	9	3	212.7	87
21	1	4	78	88	33	1.2	11	13.0	6.6	1.4	9	3	319.7	58
21	1	5	72	98	37	1.3	12	11.1	6.2	1.5	9	3	355.2	301

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
22	1	1	71	74	30	1.1	13	12.6	7.1	1.1	9	3	133.7	53
22	1	2	55	65	23	1.1	12	10.7	6.2	1.1	9	3	364.6	306
22	1	3	66	85	40	1.0	11	10.4	6.1	1.3	9	3	124.2	299
22	1	4	63	92	47	1.2	11	8.5	6.2	1.3	9	3	149.9	308
22	1	5	97	100	48	0.9	12	14.0	7.6	1.8	9	3	278.8	292
23	2	1	87	84	32	1.2	15	15.6	8.1	2.4	1	3	181.2	102
23	2	2	90	79	24	1.1	12	10.2	5.3	1.1	10	3	302.2	65
23	2	3	68	83	29	1.2	11	15.2	9.0	1.9	9	3	346.3	82
23	2	4	76	76	34	1.2	13	10.5	5.8	1.2	1	3	113.9	56
23	2	5	82	81	32	1.1	12	11.8	6.9	1.3	1	3	218.7	73
24	2	1	90	93	40	1.1	13	16.5	9.6	2.3	9	3	383.6	285
24	2	2	79	74	35	0.8	13	12.5	9.0	1.5	9	3	78.8	291
24	2	3	81	80	32	1.1	12	12.0	6.8	1.0	1	3	179.7	308
24	2	4	92	93	35	1.4	13	11.5	7.1	1.5	1	3	303.3	298
24	2	5	78	97	38	1.4	13	15.6	8.2	1.6	1	3	231.6	308
25	2	1	87	83	33	0.8	13	12.0	6.0	1.3	9	3	239.1	301
25	2	2	80	90	43	0.8	12	11.1	6.5	1.2	9	3	215.2	272
25	2	3	72	95	40	0.9	11	11.7	7.2	1.7	9	3	294.0	272
25	2	4	78	79	38	0.7	14	12.3	7.5	1.7	9	3	153.3	291
25	2	5	88	121	48	1.1	15	14.8	7.5	2.0	9	3	439.3	286
26	2	1	75	92	35	1.8	12	11.5	6.6	1.7	5	3	229.2	87
26	2	2	86	97	35	1.1	15	11.7	6.0	1.3	5	3	369.9	300
26	2	3	77	88	30	1.1	15	8.4	4.4	1.1	5	3	217.3	306
26	2	4	73	74	21	0.8	12	9.7	4.5	1.0	3	3	158.4	318
26	2	5	90	63	17	1.1	12	7.0	3.9	1.0	3	3	224.7	325
27	2	1	100	96	38	1.3	13	14.3	7.2	1.9	9	3	272.2	298
27	2	2	100	121	43	1.2	13	13.5	7.3	1.8	9	3	288.9	291
27	2	3	97	113	42	1.2	9	14.0	8.2	1.7	9	3	279.8	306
27	2	4	94	115	43	1.4	12	9.0	5.5	1.0	9	3	395.9	300
27	2	5	91	88	31	1.1	9	16.2	8.8	1.8	9	3	348.1	305
28	2	1	51	28	*	*	13	12.1	6.4	1.4	*	*	25.0	79
28	2	2	79	80	29	1.1	13	13.3	8.3	2.1	9	3	151.7	58
28	2	3	84	93	40	1.8	15	14.4	7.3	1.8	10	1	124.3	317
28	2	4	85	92	41	1.1	13	13.1	6.4	1.5	8	3	298.4	315
28	2	5	82	73	39	0.8	12	12.5	7.3	1.5	1	3	239.6	75
29	2	1	82	86	40	0.8	14	9.2	5.6	1.0	1	3	162.4	65

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
29	2	2	85	100	38	1.2	13	9.9	5.0	1.5	9	3	481.9	298
29	2	3	115	97	43	1.2	13	14.9	7.3	1.9	1	3	401.6	297
29	2	4	93	100	36	1.0	12	15.7	8.4	2.1	7	3	413.2	299
29	2	5	100	113	47	1.4	11	14.2	7.7	2.1	1	3	317.0	291
30	2	1	82	103	37	1.0	12	13.6	7.0	1.6	9	3	326.8	304
30	2	2	96	96	34	1.1	12	13.0	6.8	1.7	9	3	435.8	293
30	2	3	100	96	33	1.1	10	13.0	7.7	1.2	9	3	500.6	293
30	2	4	87	116	40	1.2	15	13.0	6.9	1.5	9	3	320.9	301
30	2	5	100	109	39	1.2	15	11.6	6.1	1.6	9	3	348.5	305
31	2	1	87	82	30	1.0	11	13.5	7.6	1.7	9	3	373.8	68
31	2	2	90	89	30	0.9	13	9.2	5.0	1.4	9	3	330.8	308
31	2	3	100	90	30	1.6	11	14.5	7.6	1.4	9	3	288.6	315
31	2	4	46	72	37	1.0	12	12.8	7.0	1.6	7	1	58.7	301
31	2	5	84	106	48	1.4	12	14.7	8.0	1.3	9	3	319.9	75
32	2	1	112	100	46	1.5	14	16.0	8.8	2.3	9	3	419.3	306
32	2	2	90	94	35	1.6	14	16.2	9.0	2.0	1	3	337.6	292
32	2	3	93	90	32	1.6	15	12.7	7.0	1.5	9	3	232.6	301
32	2	4	88	83	43	1.2	14	15.2	7.5	1.5	5	3	372.6	300
32	2	5	78	73	33	1.3	12	9.2	5.2	1.3	9	3	241.0	301
33	2	1	92	87	35	1.2	11	13.5	7.7	1.8	1	3	313.5	307
33	2	2	88	95	45	1.4	11	14.0	7.5	2.2	9	3	201.6	76
33	2	3	89	104	39	1.2	11	14.0	7.0	1.3	1	3	299.9	292
33	2	4	87	99	34	1.1	12	13.0	6.6	1.4	9	3	262.4	301
33	2	5	90	80	31	1.7	15	11.3	5.6	1.8	9	3	231.9	74
34	2	1	78	88	40	1.0	14	12.0	7.1	1.4	9	3	221.6	285
34	2	2	83	94	37	0.9	11	13.3	7.5	1.8	9	3	171.2	300
34	2	3	72	108	37	1.0	12	16.2	9.2	1.6	9	3	208.7	288
34	2	4	66	77	30	1.1	13	11.0	6.0	1.3	9	3	141.1	292
34	2	5	65	72	29	0.7	14	12.0	6.2	1.2	9	1	70.4	307
35	2	1	73	84	38	0.9	13	12.7	7.2	1.7	6	3	221.9	292
35	2	2	83	82	43	1.5	12	12.2	6.5	2.0	6	3	255.5	90
35	2	3	93	105	48	1.8	12	11.3	7.4	1.8	10	3	304.2	290
35	2	4	98	103	46	1.6	13	17.1	8.7	2.4	6	3	401.4	75
35	2	5	90	92	35	1.4	13	12.0	6.0	1.2	7	3	443.4	299
36	2	1	88	107	47	1.2	13	17.0	9.0	2.0	9	3	270.7	294
36	2	2	80	88	28	1.7	12	7.0	4.5	1.1	1	3	265.3	306

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
36	2	3	63	86	40	1.1	11	13.5	8.0	1.6	9	3	133.2	292
36	2	4	71	102	33	1.0	11	12.1	6.3	1.7	9	3	257.4	298
36	2	5	77	90	41	1.3	12	12.0	7.4	1.5	9	3	167.3	76
37	2	1	70	79	34	1.0	12	14.1	7.6	1.6	9	3	215.4	76
37	2	2	91	75	32	0.8	16	9.3	5.0	0.9	9	3	95.3	301
37	2	3	84	85	30	1.1	12	11.8	6.5	1.4	9	3	238.4	291
37	2	4	88	97	29	0.9	11	16.0	8.5	2.1	9	3	347.4	279
37	2	5	84	99	40	1.2	11	13.6	7.2	1.6	9	3	243.4	294
39	2	1	49	25	*	*	12	10.4	5.5	1.5	*	*	32.5	74
39	2	2	58	90	40	0.8	12	12.0	6.5	1.8	9	3	332.7	305
39	2	3	61	90	39	0.9	12	12.2	6.6	1.8	9	2	51.7	62
39	2	4	80	105	30	1.4	12	15.2	8.2	1.7	1	3	388.4	306
39	2	5	87	100	33	1.1	13	14.1	7.5	2.0	9	3	231.0	52
40	2	1	59	28	*	*	13	12.3	7.2	1.4	*	*	68.6	*
40	2	2	70	96	36	1.5	14	12.0	6.5	2.0	9	3	242.2	294
40	2	3	84	82	27	1.0	12	10.0	6.0	1.0	9	3	153.1	291
40	2	4	100	108	34	1.5	15	14.5	7.8	1.5	9	3	411.7	293
40	2	5	66	88	42	1.2	12	9.5	6.0	1.1	9	3	153.0	295
41	2	1	72	82	35	1.0	14	9.6	5.0	1.4	1	2	221.9	57
41	2	2	73	68	34	0.7	12	13.3	7.0	1.8	1	2	267.8	58
41	2	3	69	91	30	1.2	12	13.0	7.5	2.4	8	3	332.9	292
41	2	4	78	82	30	1.4	13	13.2	7.5	2.0	8	3	203.1	54
41	2	5	90	78	33	1.0	13	10.5	6.0	1.4	9	3	309.6	293
42	2	1	89	82	31	1.2	10	12.1	6.5	1.2	9	3	336.9	76
42	2	2	86	81	30	1.0	11	11.0	6.0	1.0	9	3	179.6	299
42	2	3	76	93	43	1.0	13	10.5	5.8	1.5	9	3	198.2	292
42	2	4	67	77	30	1.0	12	10.3	6.0	1.3	9	3	177.8	292
42	2	5	79	85	40	1.1	12	13.5	9.0	1.8	9	3	286.8	70
43	2	1	68	79	41	1.4	13	11.5	6.0	1.5	9	3	132.1	293
43	2	2	88	88	30	0.8	13	14.5	7.8	1.8	9	3	395.4	307
43	2	3	56	68	32	1.1	13	12.0	6.5	1.5	9	3	35.3	299
43	2	4	100	114	54	1.5	13	17.2	9.5	2.7	9	3	537.4	298
44	2	1	73	67	29	1.1	12	12.6	7.0	2.0	9	3	249.4	299
44	2	2	88	88	40	1.1	12	11.0	7.0	1.6	9	3	342.7	70
44	2	3	38	66	28	1.0	13	10.0	5.0	1.3	9	3	182.6	91
44	2	4	46	77	34	0.6	11	12.1	7.0	1.6	9	3	200.2	346

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
44	2	5	63	70	35	0.9	13	9.3	5.1	1.2	1	3	210.5	346
45	3	1	60	76	26	1.0	14	11.7	5.9	1.4	1	3	188.8	68
45	3	2	70	74	30	1.1	10	12.7	6.6	1.4	4	3	125.7	298
45	3	3	97	97	36	1.2	14	15.4	7.6	1.7	9	3	483.2	71
45	3	4	80	104	49	1.3	12	12.1	6.5	1.9	1	3	343.6	71
45	3	5	90	86	33	1.2	11	15.3	8.5	2.1	7	3	397.5	293
46	3	1	100	109	48	1.1	10	11.3	5.5	1.2	9	3	483.4	292
46	3	2	90	104	47	1.2	12	15.0	7.2	2.0	9	3	413.3	287
46	3	3	82	110	44	1.2	13	16.0	8.0	1.5	9	3	344.7	292
46	3	4	70	90	37	1.0	16	15.0	8.1	1.9	9	3	183.0	286
46	3	5	78	92	44	1.0	15	12.6	8.1	1.5	9	3	139.6	287
47	3	1	60	83	29	1.0	14	12.0	6.2	1.3	9	3	236.9	293
47	3	2	100	103	41	1.2	12	14.8	8.1	1.9	9	3	366.6	74
47	3	3	94	97	44	0.8	13	11.6	7.0	1.6	1	3	268.3	286
47	3	4	77	100	46	1.2	14	14.0	7.8	1.5	9	3	222.2	300
47	3	5	85	95	45	1.0	13	16.1	8.5	2.2	2	3	400.7	299
48	3	1	78	79	37	0.9	11	13.0	8.0	2.0	9	3	181.3	299
48	3	2	76	92	35	0.9	14	14.2	8.0	2.0	9	3	312.2	301
48	3	3	87	118	54	1.3	14	14.7	7.7	1.6	9	3	364.9	300
48	3	4	114	107	34	1.0	14	12.0	6.3	1.5	9	3	328.9	320
48	3	5	79	106	42	0.7	12	16.3	9.4	2.0	9	3	354.7	78
50	3	1	86	97	33	1.0	12	14.0	8.0	1.8	9	3	347.5	291
50	3	2	80	99	38	1.2	12	12.5	6.1	1.2	9	3	320.1	278
50	3	3	92	97	43	0.8	11	16.0	8.4	1.6	9	3	340.1	291
50	3	4	74	96	37	0.9	16	11.0	5.8	1.1	9	3	341.0	300
50	3	5	80	76	37	1.0	11	10.1	5.3	1.2	9	3	150.6	291
51	3	1	93	89	32	0.8	13	12.0	6.1	1.6	1	3	299.4	76
51	3	2	110	91	20	1.0	9	13.5	6.5	1.9	4	3	335.8	75
51	3	3	81	69	29	0.6	12	12.6	7.2	1.9	1	3	248.8	52
51	3	4	53	83	37	0.6	14	12.1	6.2	1.6	9	3	186.8	306
51	3	5	100	102	40	1.0	12	10.8	5.6	1.9	1	3	459.8	300
52	3	1	76	93	33	1.5	16	15.5	8.6	2.3	9	3	267.6	291
52	3	2	93	104	53	1.0	15	15.2	9.0	1.5	9	3	135.0	82
52	3	3	70	86	31	1.0	12	15.8	8.0	2.0	9	3	254.8	81
52	3	4	90	90	36	1.0	13	12.7	7.0	2.0	9	3	329.8	64
52	3	5	83	108	53	1.4	12	12.0	7.2	2.0	1	3	307.0	84

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
53	3	1	64	73	32	1.1	14	13.0	7.6	1.6	9	3	176.2	67
53	3	2	83	92	40	1.2	14	11.6	6.1	1.5	9	3	240.5	60
53	3	3	76	96	31	0.9	12	12.4	6.6	1.6	9	3	365.6	65
53	3	4	87	105	41	1.5	14	13.5	7.1	1.5	1	3	381.4	75
53	3	5	92	97	40	1.0	13	16.0	8.1	1.6	9	3	539.5	313
54	3	1	80	94	37	1.3	13	11.8	6.7	1.3	9	3	292.9	306
54	3	2	77	81	35	0.9	13	13.3	9.1	1.6	1	3	311.4	277
54	3	3	88	105	39	1.4	13	11.2	6.1	1.3	6	3	342.6	312
54	3	4	90	100	31	0.9	12	15.3	9.0	2.2	9	3	239.3	64
54	3	5	95	103	44	1.2	13	13.5	7.7	1.4	9	3	407.4	99
55	3	1	74	64	30	1.6	12	14.5	7.3	2.2	6	2	207.8	64
55	3	2	76	57	20	1.2	13	11.2	6.3	1.3	7	3	355.6	57
55	3	3	88	81	28	0.9	16	12.3	6.8	1.5	7	3	329.4	67
55	3	4	81	82	21	1.1	15	12.1	6.3	1.4	4	3	170.8	74
55	3	5	69	67	22	0.9	14	12.0	6.3	1.8	8	2	109.4	316
56	3	1	94	91	28	1.1	15	10.3	5.4	1.6	9	3	258.4	300
56	3	2	82	108	48	1.0	13	11.2	6.7	1.4	9	3	309.3	292
56	3	3	95	124	42	1.8	13	12.0	7.0	1.5	9	3	428.2	288
56	3	4	99	114	40	1.3	12	14.2	7.8	1.6	9	3	420.2	94
56	3	5	94	108	44	1.1	13	14.0	7.0	1.8	9	3	280.7	291
57	3	1	76	83	34	1.0	11	11.2	5.6	1.2	1	3	210.7	58
57	3	2	90	99	42	1.1	13	13.1	7.5	2.3	1	3	270.0	71
57	3	3	90	86	30	1.4	13	16.3	8.5	1.9	1	3	295.5	70
57	3	4	85	94	39	0.9	13	11.5	6.2	1.6	1	3	123.3	312
57	3	5	77	81	32	0.9	12	7.5	4.4	1.4	4	3	102.1	65
58	3	1	81	69	29	0.9	14	10.0	5.6	1.5	9	3	235.3	314
58	3	2	81	77	34	1.4	11	13.4	8.0	1.9	5	3	246.5	301
58	3	3	89	89	32	1.1	13	12.2	6.2	1.8	1	3	305.6	306
58	3	4	86	65	27	1.7	11	10.2	6.7	1.6	1	3	275.4	76
58	3	5	96	88	43	2.1	14	14.6	7.4	2.0	9	3	462.5	301
59	3	1	70	92	34	1.1	14	14.2	8.2	1.8	9	3	241.5	71
59	3	2	60	50	16	0.6	12	9.5	5.0	0.9	9	3	79.3	306
59	3	3	70	87	36	1.1	12	14.3	7.2	1.6	9	3	235.4	293
59	3	4	85	89	39	1.0	12	13.0	7.8	1.9	9	3	263.5	81
59	3	5	100	101	43	1.1	14	15.2	8.4	1.6	9	3	426.8	287
60	3	1	90	77	32	1.5	13	10.1	5.6	1.8	8	3	339.1	312

Plot No.	Block No.	Sample No.	Plant spread (cm)	Plant height (cm)	Raceme length (cm)	Stem width (cm)	Leaflet No.	Leaf diameter (cm)	Leaflet length (cm)	Leaflet width (cm)	Flower colour	No. of racemes	Dry matter (g)	Days to first flower
60	3	2	95	91	44	1.8	13	10.0	5.6	1.2	9	2	301.6	73
60	3	3	90	103	43	1.1	9	11.0	6.5	2.0	9	3	289.1	284
60	3	4	100	120	58	1.5	12	17.3	9.8	2.5	9	3	461.6	299
60	3	5	98	101	41	1.6	12	12.0	7.0	1.3	9	3	314.9	300
61	3	1	89	105	36	0.9	13	12.0	7.7	1.9	9	3	342.6	285
61	3	2	106	115	41	1.3	13	12.0	6.4	1.7	9	3	324.0	294
61	3	3	100	115	43	1.1	14	14.5	8.6	2.1	9	3	473.7	293
61	3	4	85	98	45	1.3	10	11.0	6.6	1.7	1	3	253.6	301
61	3	5	82	100	48	1.1	12	12.9	6.8	1.8	1	3	359.9	294
62	3	1	81	73	32	1.3	13	12.4	6.3	1.6	1	3	267.3	87
62	3	2	95	96	40	1.4	15	13.0	7.0	2.3	10	3	365.5	301
62	3	3	84	98	40	1.3	11	14.0	8.0	1.5	9	3	224.1	98
62	3	4	100	103	38	1.4	11	14.0	8.3	1.6	1	3	330.7	86
62	3	5	90	105	41	1.0	12	13.0	7.2	1.9	9	3	449.0	307
63	3	1	76	83	24	0.9	12	12.7	6.8	1.5	1	3	246.2	55
63	3	2	78	83	37	0.9	15	11.7	6.0	1.5	1	3	208.0	73
63	3	3	100	109	39	1.4	13	13.0	7.3	2.0	7	3	351.8	83
63	3	4	112	84	28	1.3	15	13.0	7.3	1.9	8	3	385.1	306
63	3	5	80	82	24	1.0	15	13.5	7.5	1.8	1	3	195.8	63
64	3	1	56	84	37	1.5	14	9.2	5.1	1.1	9	3	241.7	311
64	3	2	88	89	36	1.0	12	16.2	9.3	1.6	9	3	363.4	58
64	3	3	82	90	51	1.3	11	12.7	7.0	1.5	9	3	226.0	97
64	3	4	84	76	34	1.1	12	11.3	6.2	1.5	9	3	211.2	306
64	3	5	80	106	35	1.1	15	14.0	7.6	2.3	9	3	518.9	301
65	3	1	59	74	41	1.1	11	11.7	7.0	1.6	9	3	129.4	285
65	3	2	66	72	35	1.0	11	12.6	6.7	1.4	9	3	170.9	69
65	3	3	81	90	48	1.0	11	12.3	7.1	1.2	9	3	158.7	301
65	3	4	73	79	30	1.0	13	10.2	6.3	1.3	9	3	255.1	86
65	3	5	69	82	40	0.8	15	13.2	8.1	1.6	9	3	241.7	279
66	3	1	96	98	38	1.7	13	13.6	7.4	1.6	9	3	493.4	301
66	3	2	73	88	34	1.0	12	13.5	7.7	1.3	9	3	194.4	307
66	3	3	80	86	26	1.0	13	15.7	8.3	2.3	9	3	376.4	291
66	3	4	82	102	36	1.1	14	16.2	8.6	1.6	9	3	364.4	87